Spatial aspects of foraging behaviour in Eastern honeybees, *Apis cerana*

Submitted by Katie Hall to the University of Exeter

as a dissertation for the degree of

Masters by Research in Psychology

September 2017

I certify that all material in this dissertation which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……..

Abstract

The majority of plants in Asian tropical ecosystems depend on bee pollination. However, there is a substantial lack of knowledge of the behaviour and ecology of native tropical bees. In the present study I explored how the Eastern honeybee, *Apis cerana*, distributes its foragers in the local environment analysing waggle dances of foragers in four rural and urban locations in Kerala, South India. Similar to their well-studied close relatives, the Western honeybee *A. mellifera*, returning *A. cerana* foragers recruit nest mates through these dances communicating the distance and direction from the hive to a food source. I decoded the locations of food sources for which pollen and nectar foragers danced. The results suggest that the bees tend to forage over shorter distances as compared to the Western honeybees. Furthermore, I have found that the foraging distances, in which dancing foragers have travelled, can notably differ for pollen and nectar resources. However, there is no significant difference in the direction in which nectar and pollen foragers travel. The results also show that despite floral abundance in the proximity of the hive in the rubber plantation, foragers travelled significantly further in this location when compared to the distance that they travelled in the other locations. This may indicate that these floral resources might actually represent a nutritionally poor floral resource for the honeybees. Throughout all of the four locations, the honeybee colonies showed variable patterns of foraging distribution, focusing their recruitment towards areas which seemed to offer both pollen and nectar rewards. This is likely to be in response to the spatial clustering of their food sources, which may be a characteristic of landscapes that are dominated by human agri- and horticultural activities.

Acknowledgments

Firstly, I express my sincere thanks to my supervisor, Dr Natalie Hempel de Ibarra of the Centre for Research in Animal Behaviour at the University of Exeter for the continuous support and advice given throughout my MSc by Research. Her careful editing contributed enormously to the production of this dissertation.

I would also like to thank my second supervisor, Prof Hema Somanathan of the biology department at the Indian Institute of Science Education and Research Thiruvananthapuram, for making it possible to carry out this work in her department in India and for her guidance with experimental design.

I would like to acknowledge my fellow MSc research student Lucy Nevard at the University of Exeter, who had to put up with me for 5 months in India. It was tricky living in a country where we didn't know the customs or the language. But together we managed to muddle our way through with the help of banagram, a big thank you to you.

My sincere thanks go to Shivani, Neetash, Sajesh, Apu, Balu and the rest of the students and academics from IISER Trivandrum biology department who not only helped with the logistics of the experiments but also guided me through living in India for 6 months.

Thank you to the bee team, Raluca, Theo & Pizza, and to all the students and staff of the Centre for Research in Animal Behaviour at the University of Exeter for useful tips and keeping me motivated throughout the 2 years.

I would finally like to thank my friends and family for the support they have provided me and in particular, I must acknowledge the 'bank of Mum and Dad'.

This work is funded by the UK-India Education and Research Initiative (British Council, UK and DST, India).

Contents

Abstract…….....3

Acknowledgments……4

List of figures……...7

List of tables……..8

Chapter 1: Literature review……..9

1.1 Bee phylogeny and evolution……..9

1.1.1 The honeybees……..9

1.1.1.1 Dwarf honeybees (*Microapis*)……..……..11

1.1.1.2 Giant honeybees (*Megapis*)……..……11

1.1.1.3 Cavity-nesting honeybees (*Apis*)……..……12

1.2 Life history of the Western honeybee (*Apis mellifera*)…….……13

1.3 Foraging behaviour of the Western honeybee (*Apis mellifera*)…..……..16

1.4 Waggle dance communication in the Western honeybee (*Apis mellifera*).23

1.4.1 How do bees measure distance?.....27

1.4.2 How do bees gauge direction?.....28

1.4.3 How do bees decode dances?.....31

1.4.4 The effect of food source profitability on the waggle dance…….34

1.5 Round dances of the Western honeybee (*Apis mellifera*)……35

1.6 What does the dance behaviour reveal about the spatial foraging patterns of Western honeybee and its subspecies?.....36

1.7 Behaviour and life history of the Eastern honeybee (*Apis cerana*)…….40

1.8 Foraging behaviour in the Eastern honeybee (*Apis cerana*)……...43

1.9 Waggle dances in the Asian honeybees……45

1.9.1 Dance dialect curves in the *Apis* family……..……49

1.10 Study aims……55

Chapter 2: Research study……...56

2.1 Introduction……..56

2.2 Methods……59

2.2.1 Subjects……59

2.2.2 Study Area……60

2.2.3 Recording of the waggle dances……..63

2.2.4 Sampling……...64

2.2.5 Decoding of waggle dances……..64
2.2.6 Distance calibration of the waggle dances…….66
2.2.7 Statistical analysis……..67
2.2.8 Ethical note……..…..68
2.3 Results……..68
2.3.1 Calibration experiment……...68
2.3.2 Frequency of dances……..69
2.3.3 Spatial distribution of visited food sources……..72
2.3.3.1 Distance analysis…….72
2.3.3.2 Directional analysis……..77
2.3.3.3 Profitability of the food sources visited……80
2.4 Discussion……81
2.4.1 Dance dialect curves……..83
2.4.2 Dancing for nectar and pollen locations ……86
2.4.3 Foraging ranges of honeybees……88
2.4.4 Foraging comparison……..93
2.4.4.1 Foraging distances negotiated by nectar and pollen foragers……..93
2.4.4.2 Comparison of flight directions in foraging honeybees.....97
Chapter 3: Outlook…….99
References……101

List of figures

1. Morphological characteristics of bees in the *Apis* genus (source: Oldroyd & Wongsiri 2006)
2. Morphological characteristics of nests built by species of the Apis genus - (a) Dwarf honeybee (b) Giant honeybee (source: Buchwald et al. 2006) (c) Cavity nesting bee comb (Source: Photo by Katie Hall)
3. The honeybee waggle dance
4. The honeybee waggle dance – what the dance angle represents
5. Round and sickle cell transitional dances
6. Waggle dance transitioning and correlation to distance of food source in *A. mellifera*
7. Observation hive (Source: Photos by Katie Hall)
8. Map of field sites (Source: Google Maps, 2017; Google Earth, 2017)
9. Satellite photos of the field sites - a) Rubber plantation b) Rice paddy c) City d) Forest edge (Source: Google Earth, 2017) and ground photos by the hive (Source: Photos by Katie Hall)
10. Set up for recording waggle dances (Source: Photos by Katie Hall)
11. Still image of video (Source: Hall 2017)
12. Feeder (Source: Photos by Katie Hall)
13. Dance dialect curves
14. Frequency of dances across the day - Rubber plantation
15. Frequency of dances across the day - (a) Rice paddy (b) City and (c) Forest edge
16. Normalised frequency plot for distance categories - All locations
17. Distances decoded from waggle dances – Rubber plantation

18. Distances decoded from waggle dances – (a) Forest edge (b) City (c) Rice paddy
19. Distances decoded from nectar waggle dances – All locations
20. Distances decoded from pollen waggle dances – All locations
21. Compass plots - (a) Rubber plantation (b) Rice paddy
22. Compass plots - (a) City (b) Forest edge
23. Dance response – All locations

List of tables

1. Comparison of regression curves
2. Frequency of dances
3. Foraging ranges of different species of honeybee

Chapter 1. Literature review

1.1 Bee phylogeny and evolution

Approximately 130 million years ago angiosperms first appeared, relying on animals for pollination rather than wind dispersion (Friis et al. 2006; Oldroyd & Wongsiri 2006). Evidence suggests that one of the early visitors to these new plant forms were a group of spheciform wasps, which moved between plants to eat and in turn dispersed unknowingly the pollen on their body (Engel 2001). These early pollen dispersers predated the bees which expanded the niche, moving from varied foraging and hunting styles to complete reliance on feeding from flowering plants (Michener 2000). A mutualistic relationship was formed which has shaped the life-history of bees and angiosperms since (Friis et al. 2006; Olydroyd & Wongsiri 2006; Wappler et al. 2015). Bees diverged from the sphecid wasps 120-130 million years ago, with some becoming generalists and other specialists (Winston 1991; Engel 2001). Around 90-100 million years ago, the clade corbiculate Apidae appeared amongst the bees. The corbiculate bees had a unique morphological adaptation, a structure on their hind leg called the corbicula which allows them to carry collected pollen on the legs rather than on the body (Michener 2000). Corbiculate bees are classed into four different tribes; the stingless bees (Meliponini), the orchid bees (Euglossini), the bumblebees (Bombini) and the honeybees (Apini) (Oldroyd & Wongsiri 2006).

1.1.1 The honeybees

Within the subfamily Apinae there is only a single genus, *Apis*, the honeybees. It is considered that there are up to 10 recognised *Apis* species (Arias & Sheppard 2005). Common morphological characteristics that distinguish workers in the *Apis* genus from other tribes include: hairs that cover the eyes,

elongated marginal cell on the forewing, pollen press on the hind leg and a convex scutellum (Fig. 1, Snodgrass 1984; Winston 1991; Seeley 1995; Oldroyd & Wongsiri 2006). Honeybees also have a specific set of behaviours that makes them unique. These include high sociality, reproductive swarming, multiple mating and the one of interest in this dissertation communication of food sources through dance language (Gary 1963; von Frisch 1967; Camazine et al. 1999; Oldroyd & Wongsiri 2006).

The *Apis* genus can be split into three subgenera *Microapis* (dwarf honeybees), *Megapis* (giant honeybees) and *Apis* (cavity-nesting honeybees). Identification of a honeybees subgenera is relatively straight forward due to the large differences between their nests and body sizes (Fig. 2) (Engel 1999; Wokye 2000; Buchwald et al. 2006; Oldroyd & Wongsiri 2006).

Figure 1. From Oldroyd & Wongsiri (2006), morphological characteristics of bees in the *Apis* genus. The honey bee has 3 anatomical section: (1) head, mouthparts and sensory organs; (2) thorax, made up of muscles which control the wings and 6 jointed legs, and (3) abdomen, containing the organs responsible for digestion, stinging and circulation (Winston 1991; Seeley 1995).

a)

b)

These images have been removed by the author of this dissertation for copyright reasons

c)

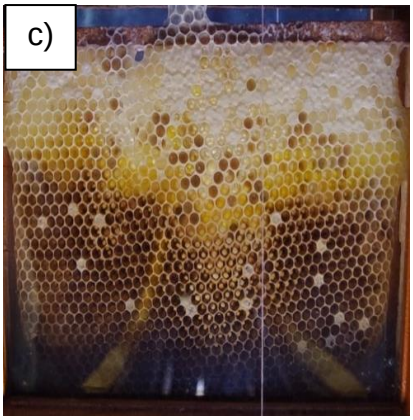

Figure 2. Morphological characteristics of nests built by species of the *Apis* genus. Photographs taken in Southern China of a dwarf honey bee nest; scale bar 10cm (a) and a giant honey bee nest; scale bar 50cm (b) both from Buchwald et al. (2006). Far right photograph of a typical comb structure of a cavity nesting bee. Combs are organised in parallel and wild colonies are usually found in hollow trees (Oldroyd & Wongsiri 2006). Photo by Katie Hall (c).

1.1.1.1 Dwarf honeybees (*Microapis*)

Dwarf honeybees are the smallest of the honeybees. They have an approximate worker body length of 7-10mm and forewing length of 6-7mm. Drones and queens can be up to 3 times the size of workers (von Frisch 1967; Rinderer et al. 1995; Oldroyd & Wongsiri 2006). They construct single comb nests approximately 10cm in diameter around a small branch (Fig. 2) (Rinderer et al. 1996). Species include *A. florea* (red dwarf honeybee) and *A. andreniformis (*black dwarf honeybee). *A. floera* has a distribution that ranges from the Middle East to Malaysia and overlaps with *A. andreniformis* distribution of the Philippines to China (von Frisch 1967; Oldroyd & Wongsiri 2006).

1.1.1.2 Giant honeybees (*Megapis)*

Giant honeybees are the largest of the honeybees with an approximate worker body length of 16.5-17.5mm and forewing length of 12-15mm. Unlike other honeybee subgenera, the wings of giant honeybees are hairy. Like the dwarf honeybees nest of the giant honeybee comprise of one singular comb.

However, their nests are it is significantly larger at approximately 50cm in diameter (Fig. 2). These nests hang on the underside of a large branch or cliff face. Species include *A. dorsata* (common giant honeybee), *A. laboriosa* (giant mountain honeybee) and *A. binghami* (Indonesian honeybee) (Oldroyd & Wongsiri 2006). The last species here is considered, by some, not to be separate species from *A. dorsata* and rather a sub-species. Phylogenetic results between *A. binghami* and *A. dorsata* have shown no clear distinction between the species with as little as 2.5% ND2 gene sequence divergence (Arias & Sheppard 2005). The distributions of species in the subgenera *Megapis* differ, with *A. dorsata* having the largest range throughout South and Southeast Asia (Oldroyd & Wongsiri 2006). *A. laboriosa* is found in the mountainous areas of Nepal (Roubik et al. 1985) particularly in the Himalayas above 1500m (Oldroyd & Wongsiri 2006). *A. binghami* is found on the islands of Butang and Sulawesi (Arias & Sheppard 2005).

1.1.1.3 Cavity-nesting honeybees (*Apis*)

Cavity-nesting bees are what is considered as the classical looking honeybee. Worker body length is approximately 10-14mm long and have a forewing length of 7-10mm. Their nests comprise of multiple parallel combs that are located inside a cavity (Fig. 2). Humans have successful domesticated bee species in this subgenus as they can be easily translocated into man-made hives and their honey is easily accessible for harvesting (Oldroyd & Wongsiri 2006). Species include *A. mellifera* (Western honeybee), *A. cerana* (Eastern honeybee), *A. koschevnikovi* (red honeybee), *A. nuluensis* (mountain honeybee) and *A. nigrocincta* (sulawesian honeybee). *A. mellifera* is the most common species of the *Apis* genus and can be found worldwide due to the movement of the species for commercial beekeeping (von Frisch 1967; Winston 1991; Oldroyd &

Wongsiri 2006). The other 4 species in this subgenus all have distributions in Asia. *A. cerana* is a diverse species and widespread in temperate and tropical Asia (Smith 2000). *A. koschevnikovi* requires rainforest habitats and has been confined to South East Asia. *A. nigrocincta* inhabits the islands of Sulawesi, Mindanao and Sagihe (Oldroyd & Wongsiri 2006). *A. nuluensis* is the least known about and is restricted to the mountainous areas of Northern Borneo, commonly at 1700m above sea-level (Tanaka et al. 2001).

1.2 Life history of the Western honeybee (*Apis mellifera*)

Western honeybees are the most studied bee species. Being a very abundant and having spread over the world by humans through beekeeping, it has attracted the attention of naturalists, farmers and scientists alike. The ecology, behaviour, social organisation, development and physiology are well understood (von Frisch 1967; Winston 1991; Seeley 1995; Goodman 2003) and its genome fully sequenced and annotated (Honeybee Genome Sequencing Consortium 2006). It is the leading model species for comparative, phylogenetic and ecological research that aims to uncover differences and similarities amongst the more than 20 000 bee species that currently exist (Roubik 1992).

A honeybee colony is considered a superorganism of bees working together to ensure reproductive success and secure the survival of their species (von Frisch 1967; Seeley 1989; Moritz & Fruchs 1997). Within a colony individuals are categorised into three different castes: queens, drones and workers. Each natural colony is formed by a queen and her offspring. Queens have a long abdomen which holds large ovaries that can produce up to a thousand eggs a day during the brood season for up to 4-5 years (Hepperle et al. 2001; Oldroyd & Wongsiri 2006). Honeybee males, or drones, remain inside the hive and are

fed by their sisters during their whole life. They leave the hive for mating trips searching for young queens, but die after a single successful mating (Gries & Koeniger 1996). The workers are the female offspring of the queen and undertake diverse tasks which include foraging, tending to the brood and nest defence (von Frisch 1967; Winston 1991; Oldroyd & Wongsiri 2006).

Caste determination happens at both the genetic and developmental level (Corona et al. 1999; Evans & Wheeler, 1999; Wheeler et al. 2006; Guo et al. 2013). The queen can control whether an egg produced is fertilised or unfertilised by regulating the release of sperm stored in her body (Camargo & Mello 1970). Fertilised eggs either grow into workers or queens. Eggs destined to be queens will be raised in queen brood cells and fed richly with royal jelly. Workers are raised in smaller brood cells and lightly fed (Brouwers et al. 1987). Drones develop from unfertilised eggs and contain only the genetic information from the queen. Workers with developed ovaries have been seen to produce unfertilised eggs and it is most common if a colony has lost its queen (von Frisch 1967; Winston 1991; Oldroyd & Wongsiri 2006).

The division of labour is based on a number of feedback mechanisms that regulate the number of bees engaged in different tasks. The worker's decision-making is influenced by social, food and brood cues (Fewell & Winston 1992; Robinson 1992; Weidenmüller & Tautz 2002). Also the bees' responsiveness to them strongly changes with their age and correspondingly with the development of brain, physiology and changes in hormonal levels (Ament et al. 2008; Schulz et al. 2002; Huang et al. 1994).

The life of an adult honeybee worker in a typical colony can be split into 3 stages. The first ten days after enclosure from the pupa, a worker spends inside

the hive performing in hive tasks, brood and queen care, and cleaning out newly vacated cells (Seeley 1995; Oldroyd & Wongsiri 2006). The feeding glands of the young worker bee fully develops after a few days. They ingest bee bread, a mix of pollen with honey and produce protein-rich royal jelly which they feed to the larvae (Free 1957). Around 10-14 days after enclosure the bees briefly leave the hive flying in front and close to it, a behaviour which is known as orientation flights behaviour. They perform orientation flights that only last 5 minutes to allow them to get accustomed to the position of the hive in the environment (Capaldi & Dyer 1999; Degen et al. 2015) (see section 1.3). Also at this age the feeding glands of the bees cease their initial function, and wax glands become active to excrete the wax for building combs. Bees of this age are also responsible for receiving and processing nectar from incoming nectar foragers, the removal of waste in the hive and guarding of the hives entrance. Finally, honeybee workers will become foragers at an advanced age of approximately 20 days (Seeley 1995; Oldroyd & Wongsiri 2006). The length of a worker's life depends strongly on the time of year. During the height of summer when foraging is high, workers only live 4-5 weeks whilst bees born late summer/autumn tend to live for several months as they can 'hibernate' during the winter season (Rueppell et al. 2007).

This succession of tasks during the honeybees' behavioural development is not strictly determined by age. Depending on the demographic situation in the colony, bee workers can become precocious foragers, nurse for longer or revert from foraging back to nursing and comb building (Robinson et al. 1992; Huang & Robinson, 1996; Amdan et al. 2005). This plasticity is controlled by social cues. When a colony is manipulated to decrease the number of foragers, younger bees that have not reached the average age for foraging start leaving

the nest and collect food (Huang & Robinson, 1996). A follow up experiment to this showed that the manipulated bees that were either forced to revert back to becoming nurse bees or become workers sooner had different levels of the juvenile hormone (JH). Bees that reverted from foragers to nurse bees have lower JH levels and consequently show a recovery of immunity with age compared to forager bees (Amdam et al. 2005). This plasticity in division of labour ensures the highest gain possible for the colony (Oldroyd & Wongsiri 2006).

Unlike other social bees and the wasps, honeybee colonies are perennial (Gould & Towne 1987). During the winter workers that enclosed in the late summer will remain inside the hive, huddle together around the queen, thermoregulate and consume their stores of honey (Simpson 1961; Fahrenholz et al. 1989; Jones et al. 2004). They leave the nest as soon as weather changes in early spring to take advantage of early flowering in plants. With fresh pollen influx new generations of workers are produced. With forage available at an increased rate at the height of the flowering season, the colonies grow too big for the old queen to dominate. Workers will rear young queens in specially built queen cells. When the first young queen emerges she kills her queen sisters and takes over part of the colony. The old queen exits in a swarm and moves to a new location that has been previously selected by scout workers. At the end of the summer, the cycle starts again (Gould & Towne 1987).

1.3 Foraging behaviour of the Western honeybee (*Apis mellifera*)

Honeybees harvest two types of food from flowers. Nectar feeds all colony members and is stored as honey. Whilst pollen is stored by the foragers,

processed into bee bread by mixing it with honey and then consumed only by the larvae and young nurse bees (Haydak 1970; Fewell & Winston 1996).

Nectar is rich in sugars, with sucrose, glucose and fructose being the main nectar sugars (Nicolson 2011). It is imbibed by actively pumping it through the bees' proboscis (Seeley 1995) and retained in the proventriculus for later regurgitation and sharing with other bees through trophallaxis inside the colony (Blatt & Roces 2002). Although the nectar collected can be directly fed to adults and brood it is mostly transformed into honey. The transformation of nectar to honey requires a two-step process. Firstly, enzymes inside the bee break down sugars in the nectar and prevents bacterial growth. Next the nectar undergoes evaporation on the tongue of the bee and when placed in the honey cells (Ball 2007). It is estimated that one larva requires 142mg of honey for its development into an adult bee (von Frisch 1967; Winston 1991).

Recently, studies have begun to reveal that other nectar elements might have various functions in regulating the nutritional state, health and behaviour of brood and workers. These include proteins, amino acids, fatty acids, minerals and toxins (Baker 1977; Baker et al. 1978; Alm et al. 1990; Adler 2000; Kim and Smith 2000; Linander et al. 2012; Nepi et al. 2012; Wright et al. 2013). The main source of proteins and fatty acids for larvae and young worker bees producing is however the pollen (Nicolson 2011). The collection of pollen is both beneficial for the plant and the bees. However, pollen varies largely because its primary function is plant reproduction. Plants have evolved strategies to limit the cost of pollen collection by bees during pollination, therefore different pollen types have different nutritional values (Nicholls & Hempel de Ibarra 2017). Once pollen has been collected it is prepared by the bees to prevent fermentation whilst it is stored long term inside the hive. When the pollen is required it is

taken out of the stores and is often referred to as ‘bee bread’ (Haydak 1970; Herbert Jr & Shimanuki 1978). This can then be consumed by both adult and larvae bees. It is estimated that one larva requires 125-145mg of pollen for its development into an adult bee (Winston 1991).

Water is a vital resource that has a significant influence on the longevity of honeybees. Experiments with queens have shown that those with restricted excess to water live two weeks less than those that have had access (Weiss 1967). Water is collected to regulate the temperature of the hive and to dilute honey. Water cannot be stored in the hive and therefore there it has a high turnover (Haydak 1970; Nicolson 2009). It is estimated that a honeybee colony requires 25 litres of water annually (Seeley 1995).

Western honeybees have evolved efficient mechanisms of division of labour and foraging that allow them to grow large-sized colony, healthy brood and reproduce successfully in many different habitats (von Frisch 1967; Seeley 1995; Winston 1991; Oldroyd & Wongsiri 2006). Essential for this is the individual forager’s ability to successfully navigate from and back to the hive over long distances. However, they have to learn the location of the hive and the views around it in order to finding back successfully. Therefore, young honeybees perform learning flights, also known as orientation flights, before they start foraging. They have several functions including calibrating with the sun, motor and sensory flight training and learning the landscape features surrounding the hive (Free 1958; Collett & Baron 1994; Capaldi & Dyer 1999; Menzel et al. 2006). As bees get older and more experienced, the number of flights decreases. The Western honeybee performs one bout of orientation flight during the morning. These flights are vital for a honeybee to safely navigate back to the nest (Capaldi et al. 2000; Degen et al. 2015).

Foragers of the Western honeybees *A. mellifera* are known to start their foraging activities in the early morning until presumably late afternoon before the light conditions become too dark or the environmental temperature too low. They regularly return to the hive to deposit food and to stay overnight. The exact timings of the commencement and finishing are very much dependent on environmental factors (Visscher & Seeley 1982; Corbet et al. 1993 reviewed by Abou-Shaara 2014). Peak times of activity seem to depend more on the availability of foraging resources than on the temperature, except when weather conditions are rainy or it becomes too hot in the middle of the day (Núñez 1966, 1970, 1982). Highest flight activity and highest rate of pollen collection have been reported to be both in the morning (Reyes-Carrillo et al. 2007; Yucel & Duman 2005) and in the afternoon (Pernal & Currie 2001).It has therefore apparent that foraging activity ranges widely due to numerous factors. These could be internal, arising from behaviour and interaction between bees inside the colony, or external determined by the natural environment, competition with other pollinators for floral resources and abundance of suitable food sources (Abou-Shaara 2014).

Foraging activity is affected by colony strength and brood rearing. With fewer bees there are fewer mouths to feed therefore the amount of food needed by the colony is much lower (Weidenmüller & Tautz 2002; Amdam et al. 2009; Abou-Shaara et al. 2013). Another in colony factor that affect the foraging activity of the workers includes the state of the queen. Colonies that have virgin queens forage as much as those that contain mated queens however they collect less pollen. Lower foraging activity and collection of pollen occurs when colonies have no queen present compared to colonies with a queen in any mated form (Free et al. 1985). Disease in the colony has also been seen to

have a significant effect on the foraging activity of honeybees. There is an increased likelihood of foragers not being able to return from foraging trips and the trips being longer when infect with *Nosema* sp. or *Varrao destructor* (Kralj & Fuchs 2006; Kralj & Fuchs 2010).

When considering external factors that affect foraging activity of the Western honeybees *A. mellifera*, weather has been shown to have a considerable impact in many countries. Higher levels of temperature and humidity have been seen to cause drops in activity levels (Núñez 1966, Gary 1967). In subtropical regions of Asia, *A. mellifera* bees were seen foraging at quite low morning temperatures, between 7°C and 16°C (Joshi & Joshi 2010, Tan et al. 2012). Average temperatures are more suited to the thermoregulatory abilities of honeybee foragers, for instance, Tan et al. (2012) report maximum flight activity of foragers at around 20°C in Asia. High temperatures restrict activities, although studies have reported foraging activity continuing in the dry European climate of up to 43°C (Blažytė-Čereškienė et al. 2010). Although, this was more than likely water collection for thermoregulation in the hive rather than foraging for food (Seeley 1995). Temperatures do not fluctuate to such extremes in the tropics, except for higher altitudes in mountains, and thus would delimit foraging activity to a lesser extent. Little is known however how foraging activity of *A. mellifera* unravels in the tropics over the day, especially around midday when peak temperatures are reached. A major weather variable that has been described are rains that dominate the wet season, but occur occasionally during the dry season and transition periods between wet and dry season. In Ghana, activity in the wet season only peaked once during early morning whilst on dry days there were up to 3 different peak activities equally spaced out in the day (Woyke 1992). Visscher & Seeley (1982) reported that in Northern America during 2

days of constant rain *A. mellifera* ceased foraging altogether. It is therefore clear that external factors, such as weather has a considerable impact in the foraging activity of *A. mellifera* and these factors should be taken into consideration when investigating foraging behaviour.

Foragers are able to use their visual, chemical and tactile senses for detection and discrimination between groups of flowers and how rewarding they are (Kevan & Menzel 2012). This allows the bees to establish foraging strategies, such as visiting foraging patches at the most optimal time of day. This would then in turn change their foraging activity and could be reflected in the peak foraging times of the honeybees (Silva et al. 2013; Abou-Shaara 2014). However toxins, including anthropogenic insecticides, usually have a negative effect on the foraging ability of honeybees (Bortolotti et al. 2003; Ramirez-Romero et al. 2005; Yang et al 2008; Decourtye et al. 2011; Schneider et al. 2012), though not always. Recent work by Pilling et al. (2013) has reported that bees foraging on oilseed rape, which has been treated with thiamethoxam, do not experience a higher risk to their health compared to a control sample of bees.

Foraging activity can be measured at the hive by the points at which foraging starts and ends (Joshi and Joshi 2010; Tan et al. 2012), the number of bees that leave, enter the hive, or net flow of bees considering both departing and arriving bees (Beekman et al. 2004; Alqarni 2006; Abou-Shaara et al. 2013). To measure how much forage bees bring back to their hive, returning foragers can be caught and their abdomen gently squeezed to extract the nectar brought back in their crop (Ramesh et al. 2016). To measure pollen, pollen traps can be used that strip the pollen sacs of the hindlegs of the returning foragers as they enter the hive (Steffan-Dewenter & Kuhn 2003). Another way to monitor activity

is to observe the foraging patches directly. This can provide information on but not exclusive to the amount of visits to the flowers (Sushil et al. 2013), the amount and type of food collected (Williams and Christian 1991) and time spent foraging (Sushil et al. 2013). More sophisticated ways of monitoring activity include the use of radio-frequency identification (RFID) and radar tracking. An RFID tag or a transponder is glued onto the thorax of a singular bee (Loper et al. 1987; Riley and Smith 2002; Decourtye et al. 2011; Tenczar et al. 2014; Thompson et al. 2016). RFID bees are then scanned as they enter and exit the hive by readers and radar bees are tracked using radar beams from on the ground satellites (Carreck et al. 1999; Van Geystelen et al. 2016).

The use of radar can not only give us an insight into forager activity but insight into the distance, direction, route and speed of the flights of foraging bees (Carreck et al. 1999; Osborne et al. 1999). For example, an experiment by Reynolds et al. (2007) found that honeybees being tracked using radar were seen to produce looping flights in order to find a previously present food source. This suggests that the honeybees were performing Lévy flight paths. *Lévy* flight theory presents the idea that there is an optimal searching strategy to locate random and sparsely distributed food sources (Viswanathan et al. 1999). Radar-tracked flight paths of honeybees were also studied by Menzel et al. (2005). The authors found that when bees were released in a familiar environment they seemed to use map-like spatial representations for setting course to a location and choosing between different goals. This and other examples exemplify the organised way in which honeybees navigate in the environment.

Radar has also been used in the examination of recruitment to food sources after watching waggle dances (see section 1.4). The results showed that even

though the waggle dance provides highly effective information on the location of the food source, visual and odour cues are required in the close proximity of the food source to accurately pinpoint it (Riley et al. 2005).

1.4 Waggle dance communication in the Western honeybee (*Apis mellifera*)

The regular behaviours performed by honeybees inside the hive which are known as ‘dancing’ behaviours have been known for a long time, with observations dating back to Aristotle’s time (Haldane 1955; Gould 1975; Tautz 1996). It was later suggested that waggle dances were linked to the bees’ foraging behaviour. Observations of bees that have returned from an experimental feeder and the recruitment of bees to a feeder indicated that honeybees could communicate to their nestmates about profitable food source (Emery 1875; Spitzner 1788; Dujardin 1853). However, it was not fully understood until the 1920’s when Karl von Frisch proved in carefully designed experiments that the dances were linked to foraging behaviour. A feeder was placed by the side of a hive and foraging bees were then marked with paint in order to distinguish her from other bees in the colony. On her return, she performs a kind of ‘round dance’ (see section 1.5) which the surrounding bees seem to be excited about and they tend to leave the nest to forage soon afterwards (von Frisch 1955). However, as the new foragers did not follow the dancing forager out of the hive, it remained unclear what information the bees transferred about the food source (von Frisch 1923).

Von Frisch was particularly interested in the waggle dances. The waggle dance is a figure-of-eight shaped movement performed by returning honeybees on the hive comb (von Frisch 1967; Grüter & Farina 2009) (Fig. 3). The bee will run at

a constant angle vigorously shaking its body from side to side at a speed of 13-15 times per second. The bee will then turn left or right and walk in a semicircle back to where it started its waggle run phase. It will then repeat the waggle run and then turn in the opposite direction and perform another semicircle walk. The amount of full circuits can range between 1-100 counts (von Frisch 1967; Winston 1991; Oldroyd & Wongsiri 2006). During the waggle dance fellow nestmates follow the dance and use their antennae to touch the bee and beg for a sample of nectar to establish how good the food source that is being advertised is (Grüter & Farina 2009).

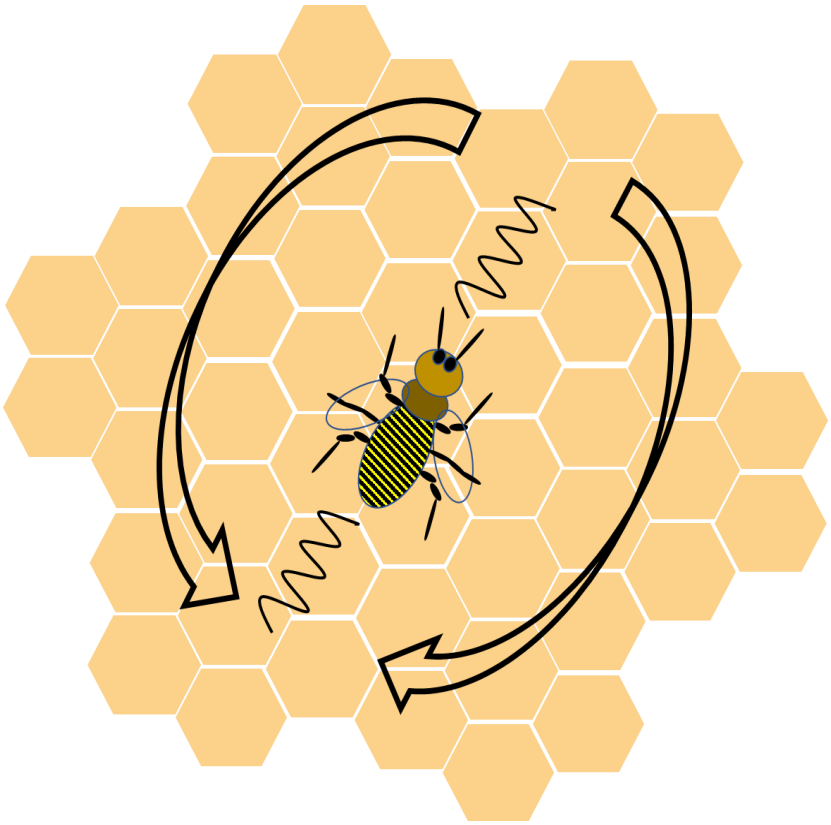

Figure 3. The honey bee waggle dance. The bee performs a straight run waggling her body and then curves left or right and walks back to the run starting position. She will then perform another run and turn the other way to walk back to the start point (von Frisch 1967). For video clip follow: https://www.youtube.com/watch?v=azt33wi51W8 (Hall 2017).

Two decades after Karl von Frisch's first observations suggesting that the waggle dance was used for recruitment to a food source, he obtained more decisive results. He trained bees to a feeder (A) and placed a second feeder (B)

further away but in the same direction as the first. Recruited bees showed up at feeder A but not feeder B which proved that the dancing bees conveyed distance to the food sources in their dances (von Frisch 1967). To confirm this result, von Frisch conducted another experiment measuring the amount of waggle runs per ¼ minute performed by bees that had fed at set feeders. He demonstrated that with increasing feeder distance from the hive, the amount of complete waggle runs decreased, i.e the dances were getting slower. This proved that there was a relationship between waggle dances and food source location. The waggle dance was being used as a form of communication to aid foraging (von Frisch 1946, 1955, 1967).

Opinions differ when it comes to the question of which part of the waggle dance codes the precise distance information between the hive and food source. It is widely accepted that distance is related to the duration of the waggle phase, however there is disagreement on whether this is duration of the waggle phase alone or the whole waggle run with subsequent turn (von Frisch 1967; Dyer & Seeley 1991; Michelsen et al. 1992).

Robotic bee models have been used to investigate the role of the different phases of the waggle dance. The models were controlled by a computer to simulate the movement, scent and sound of a real dancing bee. Different phases of the waggle dance could be manipulated in order to investigate which part is the most important for conveying accurately the information about the distance flown. Success of information transfer was estimated by measuring whether more bees were recruited to the correct feeding station (Michelsen et al. 1992; Landgraf et al. 2011). It was found that the wagging phase of the dance was the most important phase at conveying the distance to a food source (Michelsen et al. 1992).

Whilst the waggle run encodes distance to the food source, another important parameter in the dance is the communication of the direction of the food source.

To prove that the bees conveyed direction of a food source from the hive in their dances, von Frisch set up several fan experiments (von Frisch 1967; Lindauer 1971). Bees were trained to a scented rewarding feeder and dummy scented feeders were set up closer to the hive but arranged in a fan formation. Significantly more bees visited the unrewarding feeding stations that were in the direction of the feeding station. This showed that dancing bees communicated direction of the food source as well as distance (von Frisch 1967). Karl von Frisch observed that bees dancing for the same food source at the same time of day always danced with the straight run in the same direction. However, throughout the day the angle of the run changed by approximately the same degree of which the sun had moved in the sky. Therefore, the angle of which the dance is ran, relative to gravity, must be in relation to the direction of which the food source is from the sun's angle (Fig. 4) (von Frisch 1946; von Frisch 1967; Edrich 1977).

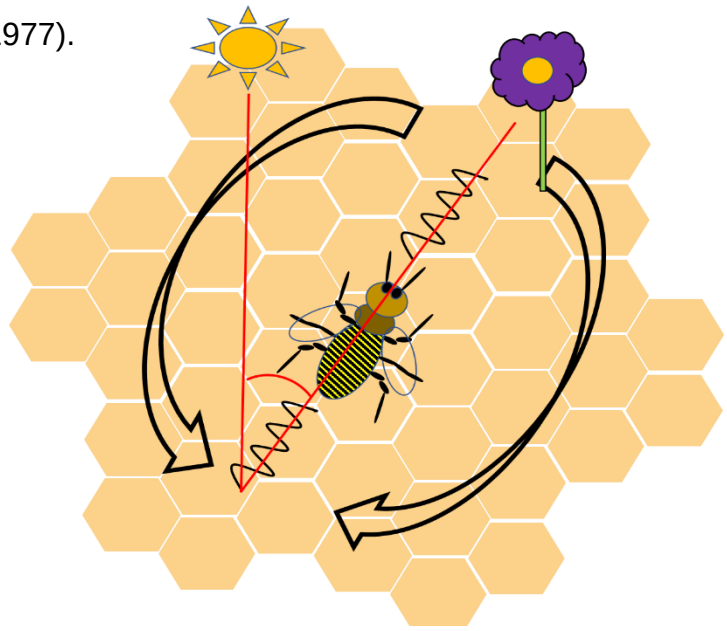

Figure 4. The honey bee waggle dance – what the dance angle represents. The red angle denotes the direction at which the food source is from the hive relative to the sun's position. The distance to the food source can be decoded from either the time taken to perform the waggle run or the time taken to perform one semicircle circuit (von Frisch 1976).

1.4.1 How do bees measure distance?

We know that honeybees convey distance information in their waggle dances, but how do they actually measure distance? Two theories have dominated the ideas about it until the early 2000s. The Energy Hypothesis proposes that distance flown is estimated from the amount of energy required for flight (Esch & Burns 1996). Observations by von Frisch found that bees flying into the wind to a feeder indicated greater distances flown compared to bees flying with the wind. This suggested that as it took more energy to fly into the wind this was the internal measure that the bees were using to gauge distance (von Frisch 1967; Esch & Burns 1996). Further work by Heran (1956) showed that bees flying to a feeder up a mountain indicated that the distance flown was a lot further than the actual ground distance. This could not be explained by flight duration or speed and supported the earlier observations by von Frisch (Esch & Burns 1996; Esch 2012). However, more recent work on looking at how energy is expended by bees has shown that bees expending more energy is not reflected in the dances, which conflicts with the Energy Hypothesis (Goller & Esch 1990; Esch et al. 1994; Esch 2012).

More convincing evidence supports another explanation formulated by the Optic Flow Hypothesis. According to this distance is gauged through optic flow, by the amount or rate of flow of image contours that are projected onto the retina of the bee. In experiments where optic flow cues were varied, it was possible to demonstrate an effect on the bees' ability to measure the distance have flown between the hive and an artificial feeder (Srinivasan et al. 1997; Srinivasan et al. 2000; Esch et al. 2001; Si et al. 2003). Bees were trained to fly inside a tunnel that was lined with vertical black-white stripes and find sucrose solution in a feeder placed at a specific location. In the tests, the width of the tunnel and

the properties of striped patterns on the tunnel walls were systematically changed to influence the optic flow that the bee experienced. When optic flow was increased, i.e contours would move at a higher rate across the retina than during training, the bee's internal odometer overestimated how far the bee had travelled and it searched further away for the feeder and danced for a longer distance in the recruiting waggle dances. When optic flow was reduced the honeybees underestimated the distance to the food source (Esch & Burns 1996; Srinivasan et al. 1997; Esch 2012). So far, there is more compelling evidence supporting the Optic Flow Hypothesis (Srinivasan 2014).

1.4.2 How do bees gauge direction?

To gauge the direction that a food source is in, bees predominately use as their navigational compass the sun and patterns of polarised skylight (Dyer 1987a). As a bee flies along its path to a food source she maintains and remembers the angle of flight from the sun's azimuth. This angle is then re-enacted during the waggle run and conveyed to follower bees (Rohrseitz & Tautz 1999; Tanner & Visscher 2008, 2009; Oldroyd & Wongsiri 2006).

Even though the sun is omnipresent, it can be a difficult landmark to use due to its movement and degree of presence (Gould 1980). These changes could be due to time, season, geographical latitude or weather (Dyer 1987a). Explanations have been put forward by Gould (1980) on how the bees compensate for the movement of the sun. The first states that the bees are programmed to calculate the azimuth as the sun moves by performing geometry. Linked with this explanation is that the bees just remember the sun's movement throughout the day and then recall the azimuth when the sun is no longer visible (Gould, 1980).

Alternatively, the bees might extrapolate the angle of the sun from the most recent observed azimuth, whether that be working backwards or forwards. This would be a good method for when the periods of time between sun movement is very small (Gould 1980; Dyer & Dickinson 1994). However, the bees could extrapolate the movement of the sun throughout the day by using the sun's average rate of movement of 15^{o} per hour (Gould 1980).

These hypothesises were tested by manipulating the bees access to the sun (Gould 1980). The authors followed this rationale: if a hive is shut off for two hours, then the rate of the sun's azimuth will change and the released forager will fly to the learned feeder accordingly to how it has compensated for the movement of the sun. For example, if a hive is closed at noon and the rate of movement is 44^{o} at that specific time then a bee extrapolating the degree from the most recent observed angle would expect the sun to move 88^{o} in two hours. Bees working on the average movement of the sun of 15^{o} per hour will expect a movement of 30^{o}. Bees working out the actual movement of the sun would work out that the sun only moved 66^{o} as past noon the movement of the sun starts to slow down (Gould 1980). To find out which hypothesis is more likely to be true Gould ran the experiment on a group of bees that were trained to a feeding station and then the hive was closed off for two hours. The hive was then moved to a new location several miles away to prevent the bees from using landmarks and forcing it to recall its vector memory for navigation. Identical feeders were then placed in the area and were monitored to determine the initial arrival area of the bees. The experiment was repeated 6 times at two different times during the day. In all the experiments, most of the bees behaved as if they had extrapolated the rate of the sun's movement from the last known rate when the hive had been shut. This resulted in either an overestimate or an

underestimate of where the sun's azimuth was. The method of extrapolation is a surprisingly simple way to compensate the movement of the sun (Gould 1980).

Dyer (1987a) argued against the hypothesis that bees could extrapolate at a linear rate from the last known azimuth position. He found that if honeybees were tested over a longer period of time, dance orientation did not occur at a constant rate but instead varied roughly similar to the sun's azimuth. This theory was later tested by Dyer & Dickinson (1994), when they restricted the bee's foraging trips to late afternoons. Subsequently, on cloudy days they would let them forage during both the morning and early afternoon. They wanted to test whether the lack of experience of celestial cues in the morning would affect the bee's orientation during their waggle dances. They found that the bees acted as if the azimuth was the starting azimuth they would have experienced if they had been let out in the afternoon. As the day progressed the bee's idea of azimuth changed at the same rate which they had experienced going from afternoon to late evening in the restrictive condition. This shows that the bees have some form of innate representation of the general pattern of solar movement rather than using extrapolations of the linear movement from the last known azimuth (Dyer & Dickinson 1994).

The effect of weather can also play a significant role in the foraging behaviour of honeybees (Riessberger & Crailsheim 1997; Henry et al. 2014). If bees were using the sun as their only way to navigate then on cloudy days navigation would be very difficult. However, as we see that bees can successfully forage on even the cloudiest of days they must be using other cues to help them navigate. One hypothesis is that the bees use local landmarks as references. Dyer (1987a) explored this theory by training honeybees along a landmark to a feeder and then moving the hive so that the landmark was in a different

compass direction. When the sun was covered by clouds bees would orientate their waggle dances as if they had followed the landmark in the original hive location. This shows that the bees had confused the sun's actual azimuth and relied on the false information from landmarks. After celestial cues were available, most bees did switch their orientation and the bees were using the correct sun position for the waggle dances. This suggests that bees can update previously stored memories of the relationship between celestial and terrestrial cues. Some bees however, reverted to their incorrect dance angle once the sun disappeared again. Other bees performed both correct and incorrect dances in different waggle runs. These observations suggest that the bees can memorise two different sets of information for orientation dependent on the location of the sun and landscape features (Dyer 1987a).

1.4.3 How do bees decode dances?

In removing the sides of an observation hive humans can easily see and decode the waggle dances of honeybees. However, when the hive is closed and in its normal state, honeybees must follow dances in the dark. How is it then possible for bees to decode dances if they are unable to see the direction of the dance and for how long it has lasted? Explanations of how the bees can follow the dances include the use of auditory, vibration, tactile or olfactory cues (Łopuch & Tofilski 2017).

The first reports that waggle dances were accompanied by sound came from Esch (1961) and Wenner (1962). They were pioneering studies but their conclusions were contradictory. Wenner (1962) agreed that sound was produced by waggling bees but could not find evidence either way that the follower bees could receive the sounds and it could convey information about a

food source. Esch (1961) on the other hand suggested that follower bees may sense vibrations of the sound using their antennae. Later Esch (1962) confirmed the importance of sound in a waggle dance by showing that bees that produce silent dances were not as successful at recruiting bees compared to the sound producing dances (Esch 2012). A further study by Towne (1985) showed that of the four most common honeybee species the two cavity nesting honeybees, *Apis mellifera* and *Apis cerana*, were the only bees to produce sound with their dances. This suggests that the sound is important for communication in dark enclosed hives (Towne 1985; Michelsen et al. 1987). Initially the source of the sound was difficult to identify, but Michelson et al. (1987) demonstrated that the sound was being produced by the wings (Michelsen et al. 1987; Łopuch & Tofilski 2017). This was later supported by Spangler (1991), Michelsen (2003) and more recently Łopuch & Tofilskie (2017). Research has also shown that when a bee has a short-winged mutation it has a 67% reduced wing area. This reduces the amplitude of sounds produced during the dance and in turn the recruitment success of the dance is reduced to less than 50%. It is now clear that sound is produced during dancing, however it is still unclear how the bees translate this sound into information about the food source location (Kirchner & Sommer 1992).

Movement of the wings not only produces sound but also vibrations on the comb. Few studies have investigated the importance of comb vibration in honeybee communication mainly due to the difficulty in measuring vibration. Tautz (1996) investigated the difference in dance recruitment between bees dancing on open empty cells compared with capped brood cells. Results showed that dancers performing on open empty cells recruited three times as many bees to a feeder compared to dancers on capped brood cells. Empty cells

caused enhanced vibration transmission but instead of the vibrations causing an increase transfer of food source information it has been shown that dances performed on open cells attract more dance followers. This proves that vibrations produced during the waggle dance are valuable at transmitting communication information to as many bees as possible (Tautz 1996; Rohrseitz 1998).

During the waggle dance follower bees frequently touch the dancing bee with their antennae. On average for 60% of the dance the follower bee have antennal contact with the waggling bee (Rohrseitz & Tautz 1999). Antennae are a multimodal sensory organ and can detect chemical, airborne nearfield and vibrations and other tactile cues from the dancing bees (Dreller & Kirchner 1993; Farina et al. 2005; Gil & De Marco 2005; Ai 2009). They are used by the follower bees to position themselves at the back or the side of the waggling bee, which have both been found as equally efficient positions to decode the location of the food source (Rohrseitz & Tautz 1999; Tanner & Visscher 2009). Touch is therefore a vital cue in communication of information during the waggle dance (Rohrseitz & Tautz 1999; Łopuch & Tofilski 2017).

Chemical signals that may play a role during dance communication include nectar and pollen taste and odours. It has been found that in some cases olfactory feeder cues may help to improve recruitment to a feeder or odour plumes are followed during a foraging trip (Johnson & Wenner 1970), however it is well established that bees prioritise the spatial information encoded in the waggle dances (Lindauer 1967; Riley et al. 2005).

Chemical signals in the form of pheromones, are frequently used in communication of activities that need to be carried out in the hive and might be

involved in the regulation of foraging activity (Slessor et al. 2005). It has been shown that a waggling bee produces and releases chemicals during the dance and when these are injected onto the comb the number of foraging bees exiting the hive increases. The findings suggest that pheromone signals could be involved in worker attraction or in modulation of foraging motivation of workers by dancing bees (Thom et al. 2007).

Odour cues from the food source are carried into the hive in the honey crop or on the body of the foraging bee (Farina et al. 2012). It has been shown that the bees can learn the odour inside the hive through associative learning forming long term memories that are likely to impact foraging decisions (Farina et al. 2005). When a hive is exposed to a scent, bees then prefer to visit a feeder that is scented with the same odour rather than an unscented source. This shows that an odour can have a significant effect on foraging preference (Arenas et al. 2008). Odour cues can then be used as an orientation guide to a food source (Wenner et al. 1969; Reinhard et al. 2004; Reinhard & Srinivasan 2009).

The dancing bee probably uses all 4 of these cues in tandem to attract follower bees to ‘watch’ their dance and to transfer the information about food source location. It is of high importance that they can get as much information across as possible to enable efficient foraging for the colony (Łopuch & Tofilsk 2017).

1.4.4 The effect of food source profitability on the waggle dance

The distance and direction or a food source is the biggest factor in determining the pattern of a bee’s dance. However, it is also affected by the profitability of the food source. With many dances occurring in the hive at once the unemployed foragers must be able to compare food sources so that they forage on the most profitable source. The profitability of a food source is measured by

several factors, including but not exclusive to: sweetness of nectar, ease of obtaining, floral fragrance, nourishment state of the colony and time of day (von Frisch 1967).

To determine how profitability is conveyed in dances, bees are trained to different sucrose concentration feeders. The resulting waggle dancers for these feeders are then analysed. It has been found that as sucrose concentration increases the number of circuits performed and recruitment increases. Dances have been seen to range between 1-100 circuits long depending on food quality (Seeley & Towne 1992; Seeley 1995; Seeley et al. 2000). Also, it is suggested that the duration of the return phase could also be a way to communicate the food profitability. Shorter return phases have been shown to indicate a source with a higher sucrose concentration (Seeley et al. 2000). Studies have also found that profitability could be shown through the vigour of each waggle run. However, it is considered that the information given through one waggle dance does not give a precise profitability measure and the bees rely on other cues such as sampling the food sources (Seeley 1995).

1.5 Round dances of the Western honeybee (*Apis mellifera*)

The round dance (Fig. 5) is a simpler form of dancing as compared to the waggle dance. It does not communicate precise information of the location of a food source (von Frisch 1967). Rather it informs nestmates that a food source is near to the hive. As the food source gets further away the round dance starts to transition into the waggle dance with a mid-phase dance resembling the outline of a sickle cell (Fig. 5). Round dances are performed when a food source is 25m away, the transitional sickle dance is performed 25-100m and then waggle

dances are performed when a food source is 100m+ away from the hive (Von Frisch 1967; Winston 1991).

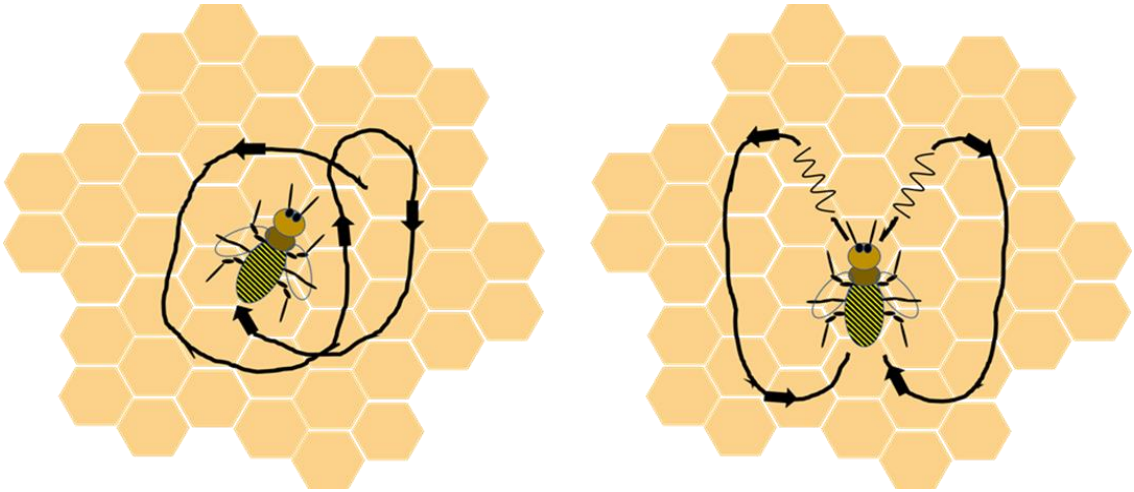

Figure 5. Left denotes the path of a bee performing a round dance. The bee repeatedly makes small circles and reverses the direction every 1-2 rounds during the dance. Sometimes complete circles are not made. Right denotes the path of a bee performing the transitional sickle dance. Small amounts of waggling occur during this dance. Both dances are followed by unemployed foragers (von Frisch 1967; Winston 1991).

1.6 What does the dance behaviour reveal about the spatial foraging patterns of Western honeybee and its subspecies?

The waggle dance has been shown to provide honeybee foragers with the information about the distance and direction a food source is from the hive. It is arguably the most sophisticated of all the insect communication behaviours. With the information that we can extract from the dances we can answer questions on how honeybees forage and construct foraging maps (Grüter & Farina 2009).

Visscher & Seeley (1982) conducted a study in a temperate deciduous forest in the state of New York looking at how over time the spatial patterns of foraging switches. They observed the waggle dances of *A. mellifera* over four 9 day periods in the summer of 1980. Bees were seen to forage regularly at several kilometres away and 95% of foraging occurred within 6km of the nest. Food source patches were exploited only for a few days and only a few number of

food patches were worked at one time. The special pattern of foraging rapidly changed and there was a strong variation in the intensity of foraging across the summer. The strong variation in foraging was attributed to the variation in foraging opportunity as this changed weekly or even daily. The colony is constantly using communication to concentrate on a small number of high value food patches and achieve optimum foraging (Visscher & Seeley 1982).

The objective of a study by Schneider (1989) was to investigate the spatial foraging patterns of the African honeybee *A. mellifera scutellata* and where possible compare it to the Western honeybee from the results in Visscher & Seeley (1982) described above. Schneider (1989) set up two colonies in the Okavango River Delta, Botswana and monitored colony one for 12 days and colony two for 4 days from August to October 1986. The dances indicated that 70-75% of food sources were 500-1000m away from the hive. Pollen dances were more common and displayed distances that were significantly shorter than nectar dances. This was likely due to increased need for pollen at the time of the experiment as brood production was at its highest. Like Visscher & Seeley (1982), Schneider (1989) found that there was a continual redistribution to foragers over numerous different food resources. However, the foraging areas and mean distances flown were significantly shorter for the African honeybee compared to the Western honey bee (Visscher & Seeley 1982; Schneider 1989). The results of this study suggest that there are differences in spatial foraging patterns between races and these are probably linked to the opposing habitats (Schneider 1989).

Waddington et al. (1994) examined the forager distributions in 2 different suburban colonies. The aim was to not only compare the two environments but also to detect differences between colonies located in the same area at the

same time. Four different colonies were equally split between 2 locations in Miami, Florida and Riverside, California for 4 and 5 days respectively. At both the locations the 2 colonies placed there had significantly different flight distances even though they were foraging in the same area. The distances were also significantly shorter than those reported by Visscher & Seeley (1982) and have been attributed to higher density of flowers in Florida and California compared to New York. For each of the locations the colonies generally foraged on different food patches but were both able to equally shift their foraging partitioning. Compared with Visscher & Seeley (1982) and Schneider (1989) however, the concentration of effort on working a small number of food patches was not as apparent. With the bees working many different patches that were more widely scattered in both Florida and California. Waddington et al. (1994) concluded that the spatial foraging patterns of honeybees varied considerable between different and within the same environment.

Steffan-Dewenter & Kuhn (2003) conducted the first experiment, to their knowledge, looking at the relationship between spatial foraging patterns and different structural landscapes. Four observation hives of *A. mellifera carnica* were routinely switched between 6 different structured landscapes in Germany. The study sites were a mixture of forest, grasslands, arable land and built up areas. They found that the month and colony significantly affected the mean foraging distances however landscape complexity did not. Variation between colonies is suggested to be due to genetic differences and differences between the months as there was a change in forage availability. The mean foraging distance of 1526m was greater than that seen in suburban areas (Waddington et al. 1994) but smaller than those seen for a temperate deciduous forest (Visscher & Seeley 1982). This indicates that there is an effect of foraging

ranges due to the structure of the landscape. There was a significant difference between the mean foraging differences of pollen and nectar foragers, with pollen foragers having to travel further than nectar foragers. This suggests that pollen was a limited resource requiring the bees to travel further for it. Also, the pollen foragers were significantly affected by the landscape, with bees foraging in simple landscapes having to travel significantly further than. The results from Steffan-Dewenter & Kuhn (2003) have given the first insight into how landscape structure can affect honeybee foraging.

Further evidence of the differences between the flight distances of nectar and pollen forages has been collected in Brighton, UK (Couvillon et al. 2015). Over 2 foraging seasons 5,484 waggle dances were recorded and decoded. Overall there were significantly fewer pollen dances compared to nectar dances and compared to previous research (Visscher & Seeley 1982; Schneider 1989). The distance travelled for pollen was also significantly shorter than flights for nectar. The foraging distance varied with month for both pollen and nectar but the variation was different for both. In some months foraging for pollen would require further flight distances then nectar and in others it would switch to nectar forages travelling further. The data suggests that forage availability is not a reason for different foraging ranges and rather it is driven by the need of one forage type over the other.

The results from these studies help us to understand how factors such as forage type, time, colony and environment influences the spatial patterns of foraging both within and comparing different species of honeybee. Future studies could then attempt to link these results to pollination, gene flow and foraging behaviour of other bee species (Visscher & Seeley 1982; Schneider

1989; Waddington et al. 1994; Steffan-Dewenter & Kuhn 2003; Couvillon et al. 2015).

1.7 Behaviour and life history of the Eastern honeybee (*Apis cerana*)

As explained in the previous sections there is a considerable amount of research conducted on the Western honeybee (*Apis mellifera*) mainly due to its abundance and availability to researchers. However, little is known about its sister species in Asia, the Eastern honeybee (*Apis cerana*) (Oldroyd & Wongsiri 2006).

Morphologically speaking the Western and Eastern honeybee are very similar and at first *cerana* were classed as a subspecies of *mellifera* (Von Buttel-Reepen 1906; Ruttner & Volprecht 1983). However later studies have shown that *A. cerana* have specific behavioural and morphological characteristics which set them apart from *A. mellifera* and prevent viable hybrids being produced (Ruttner & Volprecht 1983; Woyke 2000). It is believed that the differences between the species of honeybee are due to them having evolved in two distinct ecological niches (Ruttner 1988).

In terms of size of the Eastern honeybee is slightly smaller than its Western sister (Oldroyd & Wongsiri 2006) with forewing ranges from 7.57-9.05mm and proboscis length ranges from 4.06-6.25mm (Kshirsager & Ranade 1981; Rinderer et al. 1989; Hadisoesilo et al. 1995; Hepburn et al. 2001). The differences in sizes of the Eastern honeybee have been attributed to the large geographical distribution of *A. cerana* (Sylvester et al. 1998) as they are present in most of East Asia and have adapted to the different environments (Hepburn et al. 2001; Radloff et al. 2010; Koetz 2013). Colony size (number of bees per colony) ranges in both *A. cerana* and *A. mellifera* with the Western honeybee

tending to have larger colonies but this is highly dependent on the cavity space available (Ruttner 1988). Colonies of *A. cerana* range from 1,400-34,000 individual bees (Seeley et al. 1982; Ruttner 1988; Inoue & Salmah 1990; Karlsson 1990) and reports on *A. mellifera* have seen size ranges of 15,000-50,000 (Chinh et al. 2005; Suwannapong et al. 2012).

As stated in section 1.1.1.3, *A. cerana* is a member of the subgenera *Apis* which is the cavity-nesting honeybees. This means that they create nests of multiple parallel combs in hollow trees or in bee boxes if they have been domesticated like the Western honeybee (Oldroyd & Wongsiri 2006). Unlike *A. mellifera* though, colonies of *A. cerana* do not hibernate due to the lack of a winter and instead their behaviour is shaped by the wet season. Throughout the year, the pollen foragers, the amount of brood and pollen stored fluctuate together due to temperature and rainfall (Reddy 1980).

A. cerana has the same social structure as *A. mellifera*, as in there is a queen, female workers and male drones. Each have their specific roles in the colony as explained in section 1.2. One difference however is the higher rates of ovary activation in *A. cerana* compared to that of *A. mellifera* workers, with some reports showing that 10-20% of Eastern honeybee workers contain mature eggs (Sakagami & Akahira 1958). This figure increases to 40% 6 days after a colony has been dequeened (Oldroyd et al. 2001). The developmental steps of *A. cerana* is very similar to *A. mellifera* but occur at a slightly faster rate for brood and a slower rate for the queens (Oldroyd & Wongsiri 2006; Koeniger et al. 2011). There is also a difference between the brood nest temperatures of the two species. Tan et al. (2012) found that *A. mellifera* colonies maintain the brood nest temperature significantly higher compared to *A. cerana* and

attributed it to the need for the Western honeybee to have a higher thoracic temperature to be able to forage.

Another one of the differences between the Eastern and Western honeybee is their ability to defend against parasites and pathogens. Both species are hosts to non-parasitic and parasitic mites which either causes no significant harm or they can be extremely pathogenic, especially for *A. mellifera* (Ball & Allen 1988; Büchler et al. 1992; Eickwort 1994; Webster & Delaplane 2001; Duay et al. 2003). For example, the parasitic mite *Varroa jacobsoni* was originally hosted to *A. cerana* (Koeniger et al. 1981) but only recently has it been seen to infect *A. mellifera* (Fries et al. 1996). The recent transfer of parasites is due to *A. mellifera* being recently introduced by beekeepers into Asia as they have higher honey yields than their Eastern sister (Connor et al. 1993; Mishra & Kumar 1997; Mishra & Garg 1998). In *A. cerana* it has been found that colonies infected with the *Varroa* mite managed to remove 99% of the parasites with extensive grooming however *A. mellifera* were only able to remove 0.3% of the mites (Peng et al. 1987). Across the literature, it has been reported that *A. cerana* are more effective at removing and causing harm to mites compared to *A. mellifera* (Büchler et al. 1992; Delfinado-Baker et al. 1992; Boecking et al. 1993; Fries et al. 1996).

With differences in the morphology and life history between the Western and Eastern honeybee it is reasonable to expect that they will have differences in their foraging behaviour (Oldroyd & Wongsiri 2006).

1.8 Foraging behaviour in the Eastern honeybee (*Apis cerana*)

Like the Western honeybee the Eastern honeybee also requires workers to collect nectar, pollen and water for colony consumption (Oldroyd & Wongsiri

2006). During foraging trips bees either collect pollen or nectar but not both from one species of plant (Bakker 2001; Corlett 2011). It has been report in *A. cerana* that foragers are either nectar specialist or generalists. Generalists usually started their foraging career by collecting pollen and then shifted to nectar collection even though the demands of pollen remained constant (Ramesh et al. 2016).

The Eastern honeybee also performs orientation flights like the Western honeybee. However, preliminary data from the Eastern honeybee shows that they perform two bouts, one more than the Western honeybee. One of the learning flights occurs in the morning and the other one in the afternoon. The morning flights were predominantly carried out by older bees, while the afternoon flights were carried out by bees of all ages (Vijayan et al. 2016).

The literature suggests that *A. cerana* and *A. mellifera* have significantly different foraging activity when they are compared in the same environment (Adlakha & Dhaliwal 1979; Verma & Dulta 1986; Partap & Partap 1997; Tan et al. 2012). Foraging in *A. cerana* has been seen to start between 06:00-08:12 at ambient temperatures as low as 7°C and ceases between 17:35-19:00. Foraging activity peaks between 09:00-13:30, when the temperature ranged between 10-21°C. Length of foraging was longer and peaked earlier in *A. cerana* compared to *A. mellifera* (Verma & Dulta 1986; Verma & Partap 1994; Partap et al. 2000; Tan et al. 2012). Variations in the foraging activity of *A. cerana* are likely due to forage availability, temperature and humidity in the different geographical locations already seen in *A. mellifera* and explained in section 1.3 (Verma & Dultra 1986; Verma & Rana 1994; Partap et al. 2000).

Foraging ranges of *A. cerana* have been seen to vary among studies however generally they show to be significantly shorter than that of *A. mellifera* (Lindauer 1956; Dyer & Seeley 1991; He et al. 2013). Feeder experiments have shown maximum foraging distances for *A. cerana of* 700-1200m (Lindauer 1956; Dyer & Seeley 1991). Observations from *A. cerana* waggle dances for natural foraging sites have shown that 50% of dances indicate distances up to 195m (Dyer & Seeley 1991), 70-75% are for distances of 200-800m (Dhaliwal & Sharma 1974; Punchihewa et al. 1985) and 95% of dances show resources up to 905m meters from the hive (Dyer & Seeley 1991). Maximum distance flown has been seen to range between 1.4km (Dhaliwal & Sharma 1974) and 2.5km (Dyer & Seeley 1991).

There is conflicting results comparing the efficiency of foraging and pollination between the Western and Eastern honeybee. Verma & Dulta (1986) have reported that *A. mellifera* have significantly longer foraging trips, can carry heavier pollen loads, remained longer on individual flowers and touched more stigmas compared to *A. cerana*. Some of these differences could be because *A. mellifera* is larger than *A. cerana*. The difference in size between the two species was also what Verma & Rana (1994) concluded as the attributing factor as to why *A. mellifera* visited significantly more flowers than *A. cerana* per foraging trip in their orchard experiment. However they found that there was no significant difference between the two species for flowers visited per minute with foraging trip excluded. A contradicting study has found that *A. cerana* visits significantly higher number of broccoli flowers per minute compared to *A. mellifera* (Devkota & Thapa 2005). The differences between Verma & Rana (1994) and Devkota & Thapa (2005) are likely due to the different floral awards and their attractiveness to the bee species. It has been reported that *A. cerana*

are more attracted to apple blooms then *A. mellifera* is (Sharma et al. 2000). Time of day and stage of flowering has been seen to significantly affect the attractiveness of flowers to bees (Aly & Hassan 1999).

As expected foraging behaviour of the Western and Eastern honeybee do differ but the results are not conclusive (Lindauer 1956; Dyer & Seeley 1991; Verma & Rana 1994). *A. mellifera* has been introduced into Asia as it produces more honey than *A. cerana* (Mishra and Kumar 1997). Therefore, it is important to determine the foraging behaviour of these two species in the same environment as there is potential for resource competition (He et al. 2013; Koetz 2013).

1.9 Waggle dances in the Asian honeybees

Waggle dances have been studied to a much lesser extent in the Asian honeybee, *Apis cerana*, and other *Apis* species (Lindauer 1956; Dyer & Seeley 1991; Tan et al. 2008).

The previous descriptions of the waggle dance in this review (section 1.4) have focussed on the western honeybee, *A. mellifera*. Like the Western honeybee, the Eastern honeybee also performs waggle dances in the same fashion in their cavity nests (Dyer & Seeley 1991). The attention of research has focussed on the Western honeybee A. mellifera, and to date very little is known about the intricacies of dance behaviour in the Eastern honeybee, *A. cerana*.

It is often assumed that the dances of the Western and Eastern honeybee are identical, given their close phylogenetic relatedness and similarity of major features (Lindauer 1956; Dyer & Seeley 1991; Dyer 2002). New supporting evidence that dances of closely related *Apis* species encode information in the same way comes from recent experiments in an area of China with subtropical climate where the ranges of *A. mellifera* and *A. cerana* overlap. Both species

were reared together in the same hive. It was observed that foragers from both species cross-communicated via waggle dances efficiently even though they have different dance dialects (Su et al. 2008; Tan et al. 2008). Nevertheless, evidence is still lacking that the waggling phase of the dance for *A. cerana* is identical to that of *A. mellifera*. Therefore to date, studies decoding the waggle dances of *A. cerana* have used the whole waggle circuit for the estimation of distance (Lindauer 1956; Dyer & Seeley 1991), and despite the above-mentioned recent studies with mixed-species colonies, more research is needed to strengthen our understanding of the waggle dance behaviour in *A. cerana*. For now, we rely on the classical theory by von Frisch (1967) assuming similarity of dances between closely-related *Apis* species (Dyer 1992 review).

Species in the other 2 subgenera also perform waggle dances but as their nests have different configurations their waggle dances are slightly different and far less studied (Lindauer 1956; Dyer 1985 a, b, 1987b; Dyer & Seeley 1991). Previously it has been thought that these bees performed primitive dances compare to that of the subgenera *Apis* however it is now understood that they can communicate the location of food just as well as *A. mellifera* and *A. cerana* (Lindauer 1956; Dyer 1987b).

The description of the dwarf honeybee waggle dance only applies to *A. florea* as it hasn't been recorded for *A. andreniformis* (Oldroyd & Wongsiri 2006). On return from a foraging trip bees of the species *A. florea* perform waggle dances on the horizontal plane of the crown of the nest (Lindauer 1956; Koeniger et al. 1982; Dyer 1987b). During the waggle run of *A. florea* she holds her wings out and abdomen up and produces no accompanying sound unlike the Western and Eastern honeybee (Towne, 1985). Follower bees stand further back from the dancer compared to that of cavity nesting bees. This is suggested to be

because the dance isn't performed in the dark and therefore less physical contact is required and replaced by visual cues. As the bee can dance on a flattish surface the angle of which she is dancing in points exactly to the food source rather than the angle from the sun (Dyer 1985a; Oldroyd & Wongsiri 2006). The influence of the sun in the dances of *A. florea* has been demonstrated in the use of mirrors to distort the sun's position. Bees were housed inside a room with their only view of the sun being a mirror image that the scientists could manipulate. The bees could feed on a scented sucrose solution from a feeding dish outside of the closed room. When the mirror was moved through 45^{o} the dancing bees turned the direction of their dance through 90^{o}. This has shown that the *A. florea* use the current position of the sun to orientate their dances (Koeniger et al. 1992). With the sun being such an importance reference for the bees, cloudy days could leave them vulnerable (Rod-im et al. 2015). Dyer (1985a) conducted an experiment where a hive of *A. florea* were housed with no view of the sun from their nest and instead they were presented with a striped board. The bees could feed form a sucrose supply outside of their nest. On return the dance direction of the bee could be manipulated by 180^{o} when the striped board was moved by the same angle. This suggests that the bees were orientating their dances to this board and therefore can use terrestrial landmarks to help orientation during the absence of the sun (Dyer 1985a).

Unlike the nests of the dwarf honeybee, the nests of the giant honeybee do not provide a horizontal surface for performing waggle dances on. Therefore, dances occur on the vertical comb and dancing bees cannot point in the direct direction of the food source (Lindauer 1956). Instead they follow the same guidelines of conveying direction as the cavity nesting bees. They waggle at

angle of which the food source is relative to the current sun's azimuth. It was first thought that giant honeybees did not produce sound whilst dancing (Towne 1985), however the sound produced is such a low frequency that it was not picked up by the recorder used in the original experiment (Kirchner & Dreller 1993). Of the giant honeybee species *A. dorsata* have been shown to produce dance sounds during the waggling phase of the dance (Kirchner & Dreller 1993). *A. dorsata* can produce sounds of 90-140Hz (Kirchner & Dreller 1993) and in this range, they can learn and memorise the sound to locate a food source (Dreller & Kirchner 1994). The explanation to produce sound comes from the fact that *A. dorsata* forage and dance on moonlit nights (Dyer 1985b). In fact, during the night dances there is an almost 3 times higher chance that sounds will be produced by the nocturnal foragers suggesting that the lack of light is the driving force for producing sound (Kirchner & Dreller 1993). This is further supported by examining the dances of the mountain dwelling species of the giant honeybees, *A. laboriosa*. Due to low temperatures at night, these bees only forage during daylight hours. It was found that *A. laboriosa* do not produce any sound during their dances. This supports the theory that sound production during dances is an evolutionary adaption to facilitate the transfer of information during low light intensities (Kirchner & Dreller 1993; Kirchner et al. 1996). With sound being produced during the waggle run stage the bees can gauge how long the waggle run is and therefore how far the distance is to the food source (Wenner 1962; Dreller & Kirchner 1994).

As explained, different races and species of honeybees that have had different evolutionary development and therefore they have each established their own distinct waggle dances. This could mean there are differences in the dance languages or dialects of the different species (Gould 1982).

1.9.1 Dance dialect curves in the *Apis* family

Dance dialects differ in two ways; the progression from a round dance to a waggle dance and how time of a dance relates to a set distance of a food source (Sarma et al. 2004). The later of these differences produces dance dialect curves, which show the relationship between the average time of the waggle run or dance circuit and the distance flown (Lindauer 1956; Dyer & Seeley 1991). These dialect curves can then easily be compared between different honeybee races and species (Lindauer 1956; Boch 1957; von Frisch 1967; Dyer & Seeley 1991).

Boch (1957) undertook, under the instruction of von Frisch (von Frisch, 1967), the first systematic study of the dance dialects of the European races of *Apis. mellifera* and available *mellifera* races outside of Europe (Fig. 6). The bee races included; *A. m. carnica*, *A. m. mellifera*, *A. m. intermissa*, *A. m. caucasica*, *A. m. ligustica,* and *A. m. fasciata.* Hives were set up in the same environment and bees were trained to feeding stations. After training the feeders were set up at various distances from the hive. At the set distances, the waggle dances were observed and it was determined what type of dance they were performing; round, transitional sickle cell or waggle dance. Dances were only classed as a certain dance type if a predetermined percentage of complete dance forms was completed (Boch 1957). All bees performed unoriented round dances, however the range of performance differed with the distance to the feeder. In the order of bee races presented earlier, round dances persisted from further to shorter distances. In other words, *carnica* performed round dances for the longest with the maximum of 15m away whilst *fasciata* performed round dances the shortest up a maximum of 2m away. The range of the transitional sickle cell dances was characteristic to each bee race. *A. m. carnica* did not perform transitional sickle

cell dances at all and instead transitioned from the round dance to the waggle dance. The start of the waggle dance began first with *fasciata* at feeders set at 12m away from the hive and *carnica* was the last race to start to perform waggle dances at feeders placed at 85m away (Boch 1957; von Frisch 1967). In figure 6 the transition from one dance form to the next are shown (Boch 1957; von Frisch 1967).

Figure 6. From von Frisch (1967) showing results from Boch (1957). Left shows the distances at which dance types transition. R: Round dance, S: Transitional sickle-cell dance and T: Waggle dance. Right shows the dialect curves for races of *A. mellifera*. The result from *A. mellifera adansonii* comes from Smith (1958).

Boch (1957) also examined the indication of distance for the races over a range of 100-500m and supplemented his results with that of Smith (1958) who observed the dance rhythm of *Apis m. adansonii*, which is a race of honeybee from tropical Africa (von Frisch 1967). The race that performed the most rapid dance rhythm were the *carnica* and the slowest dancers were the *fasciata* and *adansonii* (Bosch 1957; Smith 1958; von Frisch 1967). In figure 6 you can see dance tempo plotted against distance to feeding site, the data of which has been taken from Boch (1957) and Smith (1958) but created by von Frisch (1967). These are commonly known as dance dialect curves and it suggests that there are differences in the dance dialects of races of *A. mellifera* (Boch 1957; von Frisch 1967).

Given the differences in dance dialects between races of honeybees, it is reasonable to expect that there would be differences between species of honeybee as they are morphologically very different (von Frisch 1967). Lindauer (1956) provided the first conclusive report on the communication system of Asian honeybees. It was found that all three of the Asian species perform round and waggle dances like that of the *A. mellifera*. However, these round dances converged quickly onto waggle dances once the food source was moved further away from the hive. There are variable results in at what distance away from the hive the transition between dance types ends and begins (Lindauer 1956; Sakagami 1960; Towne & Gould 1988; Sarma et al. 2004). Lindauer (1956) reported that dwarf honeybees, *A. florea*, shifted their round dances to waggle dances at feeders over 5m away from the hive and giant honeybees, *A. dorsata*, shifted at feeders placed at distances over 3m. Towne & Gould (1988) reported convergence onto waggle dances at closer distances with *A. cerana* at 5m, *A. dorsata* at 2.5m and *A. florea at less than 1m.* However, experiments with the Japanese *A. cerana* reports that the transition to waggle dances is much longer than previously stated (Sakagami 1960). Also, a comparison between *A. florea* and *A. mellifera carnica* has shown that there was no significant difference between the proportion of round and waggle dances at the same feeder distances and no significant difference between the time point at which transition between the two dance types was made (Sarma et al. 2004).

Another aspect of dance dialect differences between species is the relationship between circuit and/or waggle duration and the distance it corresponds to. The first studies by Lindauer (1956) and Punchihewa et al. (1985) in Sri Lanka showed that the Indian species *A. cerana*, *A. dorsata* and *A. florea* have significantly different dance dialect curves. *A. dorsata* have the fastest dances

followed by *A. cerana* and *A. dorsata*. When Dyer & Seeley (1991) tried to replicate these experiments in Thailand they unexpectedly found that there was no significant difference between the three Asian species' dance dialects. When comparing the same species' slopes in Sri Lanka (Lindauer 1956) and Thailand (Dyer & Seeley 1991) they found that they had significantly different dialect curves in all 3 species but less pronounced in *A. dorsata*. Comparisons of the dialect curves from Sri Lanka and Thailand also show that in Sri Lanka the dialect curves clearly diverge between the 3 species whilst they are quite parallel in Thailand (Dyer & Seeley 1991). The differences between the two countries would suggest that there are some regional dialect differences in the Asiatic species comparable to what has previously been reported by Boch (1957) in *mellifera* (Dyer & Seeley 1991).

Lindauer (1956) and Punchihewa et al. (1985) also have added comparisons with previous *mellifera* studies (von Frisch 1951; Schweiger 1958) to their data and both agreed that races of *A. mellifera* have similar dance dialects to the Asiatic honeybees but are most like *A. dorsata* in the way that they decline at a slower rate. However, these *mellifera* studies were not completed under the same protocol, location or time of year. It is uncertain whether these differences were due to species differences or external variables (Su et al. 2008). Su et al. (2008) therefore undertook a study in China where the waggle dances of *A. cerana cerana* and *A. mellifera ligustica* could be directly compared in the same environment. They found that there was a significant difference between the dance dialect curves of the two species, even when the foraging environment was the same. These results suggest that there is a lot of variation between the dance dialects and brings to question what external or internal factors affect them (Su et al. 2008).

For many years, it was widely accepted that the dialect curve was influenced by natural selection to reflect the typical foraging range of the species (Lindauer 1957; Boch 1957; von Frisch 1967; Gould 1982; Oldroyd & Wongsiri 2006). This was called the adaptive tuning hypothesis and states that there are two factors that have influenced the evolution of the dance dialect. Firstly, the shorter the foraging range of the bee the steeper the dialect curve because steep dialect curves are more precise over shorter distances. The more precise a dance can be the less distance error there will be when a bee is foraging for a food source. The second factor involves the length of the dance. Where a bee has a large foraging range it would be an insufficient use of time and energy to have slow dances at long distances. Therefore, bees that have large foraging ranges would benefit from having shallow dialect curves (von Frisch 1967; Dyer & Seeley 1991).

Lindauer (1956) and Punchihewa et al. (1985) supported the adaptive tuning hypothesis with their foraging ranges and they found that the bees with the longer foraging ranges did indeed have shallower dance dialects. They attributed the foraging ranges to the size of the bees as the bigger the bee the larger the foraging range. The results from Dyer & Seeley (1991) did not support the adaptive tuning hypothesis. Their results showed that whilst there was a difference in the foraging ranges of the honeybees the dance dialects of these bees were very similar. Also, they did not see the relationship between bee size and foraging range. The smaller *A. florea* (max. 15.2km) had a larger natural foraging range compared to that of the larger *A. cerana* (max. 2.5km). Recently a large-scale review of 62 bee species has been undertaken to establish if there is a relationship between body size and foraging range. By using maximum homing distance, typical homing distance, maximum feeder

training distance, and maximum communication distance as estimates for foraging distances. They found that there was a positive non-linear relationship between foraging range and the distance between wing bases of the bee (Greenleaf et al. 2007). The downside of the study by Greenleaf et al. (2007) is that the bees were not tested under the same experimental conditions. As stated early bees gauge distance by the amount of optic flow that they encounter whilst flying (section 1.4.1). In different environments, the optic flow will be different and therefore could affect the dance dialect curves (Srinivasan et al.1997; Srinivasan et al., 2000; Esch 2001; Si et al. 2003; Sarma et al. 2004). Dyer & Seeley (1991) did compare the bees in the same environment but only 1 colony was used for each species. Therefore, it is still open for debate whether the adaptive tuning hypothesis is correct or if other factors are influencing the dance dialects of honeybees (Dyer & Seeley 1991; Sarma et al. 2004).

Other factors that have been proven to affect the dance dialects of honeybees involve genetic and environmental factors (Schricker 1974; Rinderer & Beaman 1995; Johnson et al. 2002; Sarma et al. 2004). Rinderer & Beaman (1995). Johnson et al. (2002) studied the genetic control in *A. mellifera* races for dance dialects. By crossing colonies that have different dance dialects they recognised that one dialect was dominant over the other. They concluded that the transition points from round to sickle and sickle to waggle dances is controlled by a single locus with more than one allele. Therefore, genetic inheritance could be a founding factor in the differences in dance dialects (Rinder & Beaman 1995; Johnson et al. 2002). Schricker (1974) investigated the effect of insecticides on dance dialect variation. *A. mellifera carnica* were trained to a feeder and control dances were observed. They were then given a sublethal dose of Parathion and

the resulting waggle dances underestimated the distance to the feeder which was seen in increased dance rhythms. This shows that external factors can influence the dance dialects of honeybees (Schricker 1974).

1.10 Study aims

The study of the literature shows that very little is known about the foraging and spatial behaviour of the Eastern honeybee, and its differences and similarities with the closely related sister species the Western honeybee (Lindauer 1956; Punchihewa et al. 1985; Dyer & Seeley 1991; Su et al. 2008). The Eastern honeybee is a vital pollinator of agricultural crops in tropical and subtropical Asia, and much of the food productions relies on their pollination services. Therefore, there are good scientific reasons as well as an economically motivated interest for learning more about their foraging behaviour, especially as pollinator populations have and continue to decrease rapidly due to human activity and habitat impairment (Partap & Verma 1994; González-Varo et al. 2013; Nayak et al. 2010).

The present study aimed to primarily investigate how foragers of *A. cerana* distribute in human-impacted landscapes of tropical South India. The findings were compared to previous work conducted with *A. mellifera* to evaluate whether inferences can be made from the closely related Western honeybees to tropical Asian honeybees. This work provides essential evidence that will inform future research asking how landscape features, of human-impacted habitats in tropical Asia, influence spatial patterns of forager movement and thus plant pollen and gene flow through pollination services provided by native tropical bees.

Chapter 2: Research study

2.1 Introduction

The majority of wild plants and agricultural crops require pollination by insects for improving their reproduction and sustaining their populations under changing environmental conditions. Bees in particular contribute vastly to pollination services making them ecologically and economically important agents in plant-pollinator networks. It is therefore important to understand the foraging behaviour of bees in order to protect the ecosystem services and promote food security (Klein et al. 2007; Garibaldi et al. 2013). .

Observations of waggle dances in honeybees offer insights into the decisions that nectar and pollen foragers make. Waggle dances are movement sequences along a figure-of-eight trajectory that foraging honeybees perform on the hive comb (von Frisch 1967; Grüter & Farina 2009). When walking straight the bee shakes its body producing a vibration signal that recruits other foragers and attracts hive bees (Tautz 1996). At the end of the run she then turns left or right and returns to the start point of the straight run. Worker bees in the hive follow the dance and it seems like they take in information from the figure-of-eight movements provided by the dancing bee (von Frisch 1967; Winston 1991; Oldroyd & Wongsiri 2006). The information that is communicated by the dancer was investigated in much detail by Karl von Frisch, his students and collaborators in the 1940s to 1960s. He demonstrated that the time taken to complete the dance was correlated with the distance between the hive and the food source. The angle of the straight run relative to gravity changed depending on the direction in which the food source was located relative to the hive and the sun's azimuth. Thus, the information that is communicated by forager bees can be decoded to inform us where the bees are foraging at any given time and

which locations in the environment have rich and abundant flowers (von Frisch 1946, 1955, 1967).

The waggle dances of the western honeybee (*Apis mellifera*) have been widely studied. Western honeybees tend to forage away from the hive, over distances of several kilometres. Visscher and Seeley (1982) found that 95% of the bees in a temperate forest in the state of New York foraged within 6km of the hive. In this and another study by Steffan-Dewenter and Kuhn (2003) conducted in Europe, the researchers found that 50% of the bees danced for closer locations, only 1-1.5km away from the hive. Many studies have also provided examples for the flexibility with which these bees can redistribute their workers when food availability or the nutritional requirements of the colony changes (Visscher & Seeley 1982; Schneider 1989; Waddington et al. 1994; Steffan-Dewenter & Kuhn 2003; Couvillon et al. 2015).

Most research to date has focussed on examining the foraging behaviour of the western honeybee (*Apis mellifera*), with a few exceptions (Lindauer 1956; Dyer & Seeley 1991; Tan et al. 2008). There is a significant lack of knowledge known about foraging behaviour in other honeybee species. Here I analysed the waggle dances of Eastern honeybees (*Apis cerana*) to investigate how pollen and nectar foragers are distributed in the environment. Studies in the tropical forests of Sri Lanka and Thailand suggest that the Eastern honeybee travels over much shorter distances when collecting food from flowers (Lindauer 1956, Dyer & Seeley 1991). They found that 50% of dances signalled foraging locations that were within 200m of the hive. Nearly all foraging locations (95%) were at distances below 1km. To study this further, I recorded and analysed the dances of Eastern honeybees foraging in the tropics of South India, in Kerala's low land. A large proportion of wild plants and agricultural crops in tropical Asia

are pollinated by this bee species (Partap & Verma 1994; González-Varo et al. 2013; Nayak et al. 2010). It is popular with farmers who extract honey and often keep managed colonies on their land. It was therefore feasible to observe the dances in three rural locations where the bees had vast resources of agricultural crops and in one peri-urban location that was dominated by gardens and fruit and coconut trees as garden and street trees. I expected to find that the Eastern honeybee would forage over short distances, similar to colonies in the middle of dense forest. However, the distribution of food sources is altered in these human-altered landscapes with local differences observed in each of the locations. It could well be that foraging for nectar and pollen could lead to distinct dissociations in the distances and directions over which the bees foraged.

2.2 Methods

2.2.1 Subjects

Experimental bee colonies of *Apis cerana* were provided by a local beekeeper and certified bee breeder in Kadakkal, Kerala. These bees were bred from colonies taken from the wild. They were housed in purpose-made rectangular 2-frame observation hives with glass walls to enable the filming of returning bees performing the waggle dance (Fig. 7). Colonies consisted of an estimated 1000 bees with a single mated queen. Hives were placed on level ground on greased stands 1m from the ground to prevent invasion from ants. Where possible they were placed in shaded areas to prevent over-heating. Bees were in good health and actively foraging for nectar and pollen. Throughout the experiment no colonies performed reproductive or relocation swarms.

Figure 7. Side view of the observation hives.
Photo by Katie Hall.

2.2.2 Study Area

Data were recorded between April-June 2016, during the dry season until the start of the monsoons, in the four different habitats in Kerala, a state in the South-West of India (Fig. 8). Besides logistical considerations and access to private land or buildings in the city, the four locations represent typical areas with high level of fragmentation due to human agricultural and settlement activity. They are characteristic for human-impacted areas in Kerala that continuously expand, reduce and fragment natural forests in the Western Ghats. Two locations were in the middle of an agricultural area dominated by tree plantations, fields and small settlements (rubber plantation: 8°50'09.65"N, 76°54'23.00"E; rice paddy: 8°51'24.59"N, 76°56'33.81"E). The third location was in the peri-urban area of Trivandrum, the capital of Kerala, in an area dominated by house gardens and city trees (city: 8°32'38.51"N, 76°55'20.13"E). The fourth location was in a rural area with a landscape mix of forest fragments, fields and small settlements (forest edge: 8°52'26.89"N, 77°06'31.20"E) (Fig. 9). Due to logistic difficulties of sourcing new hives and rehousing them in the observation hive box in time before the start of monsoons, the same hive was moved from the rubber plantation to the rice paddy. They were given at least 5 days to adjust their foraging patterns to the new location.

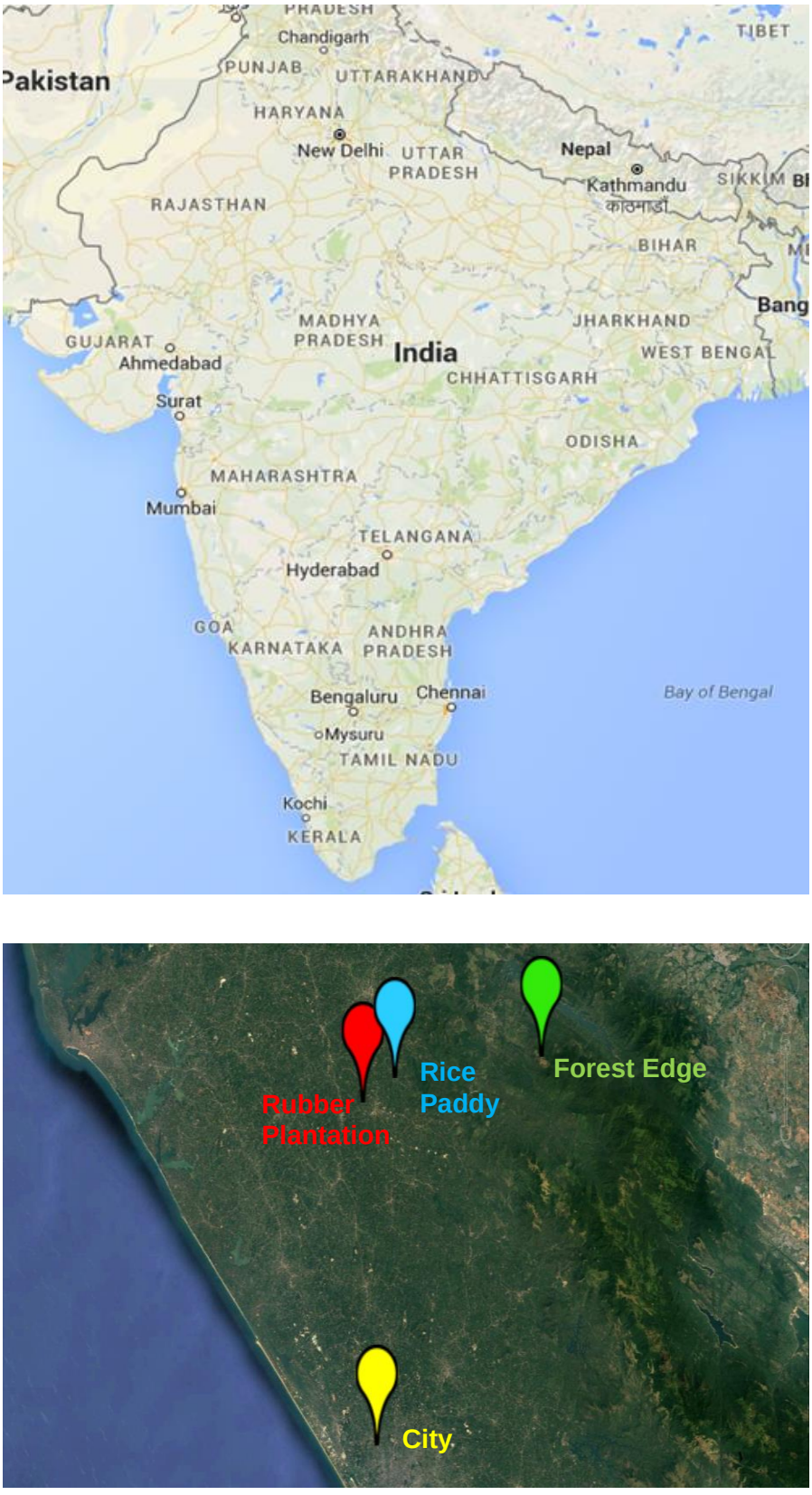

Figure 8. Map of India. State of Kerala is on the south west coast (Google Maps, 2017). Satellite photo bottom (Google Earth, 2017) of the field sites, marker placement shows the location of the hive. Red: Rubber plantation, Blue: Rice paddy, Yellow: City and Green: Forest.

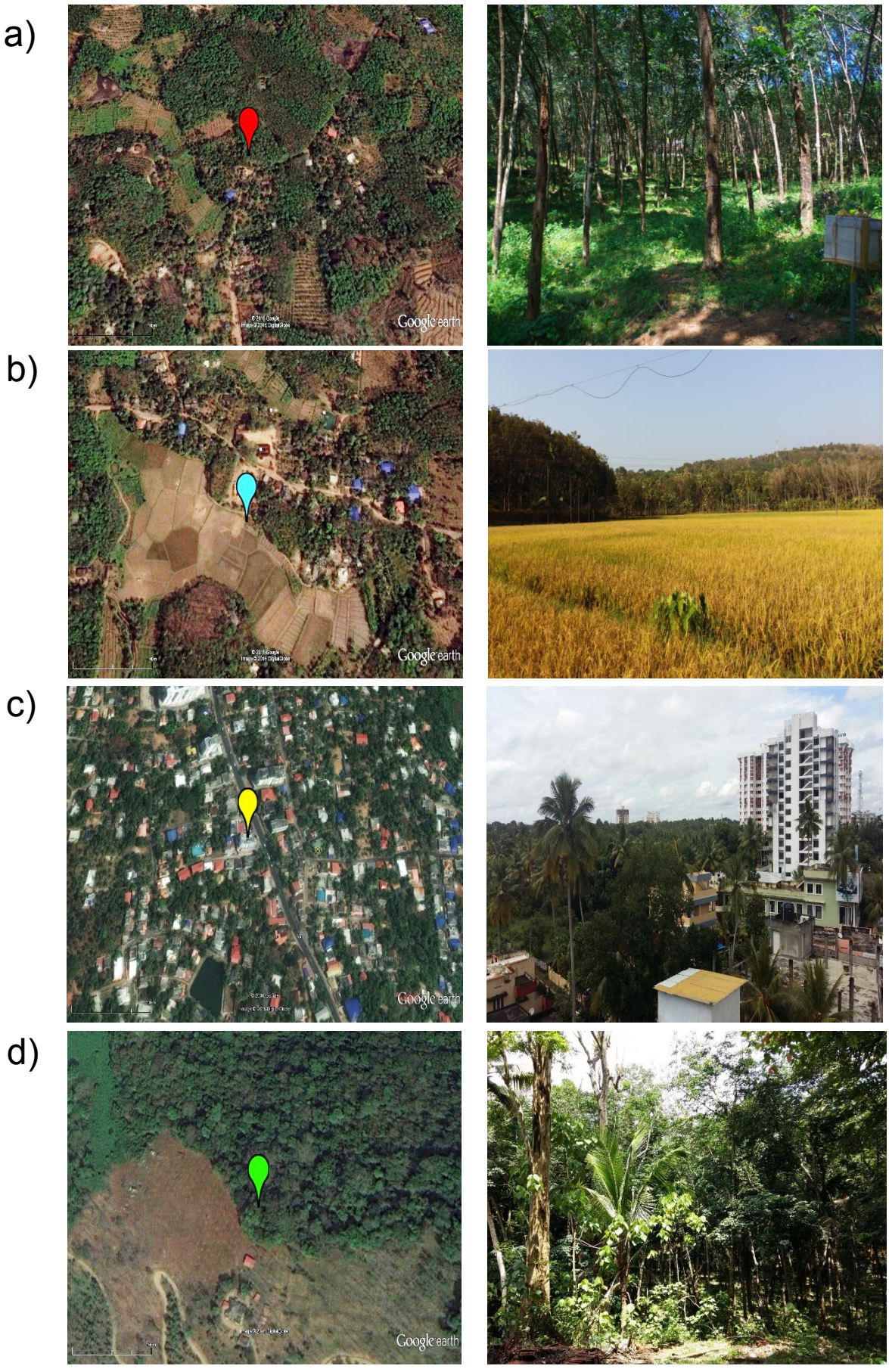

Figure 9. Left shows satellite photos of the field sites (Google Earth, 2017), marker placement shows the location of the hive. Right shows ground photos by the hive. a) Rubber plantation b) Rice paddy c) City d) Forest edge. Photos by Katie Hall.

2.2.3 Recording of the waggle dances

Dances were recorded between 06:30 and 13:30. It was not possible to film dances during any of the afternoons due to rainy weather conditions. The hive was filmed from both sides simultaneously using a Pentax K-500 camera (HD, 30fps) and a Sony Handycam HDR-CX410 (HD, 25fps). To reduce glare, a black cloth was placed over the hive which provided shade and kept the hive cool (Fig. 10). Only the lower part of the hive near the entrance was filmed given that I observed that the bees would dance mainly in this area. Also previous experiments in *A. mellifera* have shown this lower area of the comb to be the dance floor (Körner 1940; von Frisch 1967). In one experiment 94% of *A. mellifera* dances occurred within 24cm of the hives entrance (Seeley & Towne, 1992). A heavy screw on a length of string was hung from the top of the hive as a reference for measuring dance angles as the bees dance in relation to gravity (Fig. 11).

Figure 10. Left shows the recording of the hive on both sides and rights shows the final set up with cloth covering to prevent the hive overheating. Photos by Katie Hall.

Figure 11. Still image of an example video recorded. The white line is the reference string for gravity. Photo by Katie Hall. For video clip follow: https://www.youtube.com/watch?v=azt33wi51W8 (Hall 2017).

2.2.4 Sampling

In total 2242 dances were detected from an estimated 290 hours of video footage. None of these dances presented as that of the modified dance language associated with absconding/migrating in *A. cerana*, therefore all were assumed to be dancing for food sources (Sasaki et al. 1990). All pollen dances were decoded (n=328), as their number was smaller. A predetermined number of nectar dances were systematically sampled across the day taking into consideration the frequency of dances in each time slot (n=409). This predetermined number was determined by how many pollen dances there were in that location so that the dances of each forage type could be compared.

2.2.5 Decoding of waggle dances

Dances were decoded using Adobe Photoshop CC 2015.5. The waggle dance is an easily distinguished behaviour from the resting background bees in the nest. Once identified, the video was rewound to when the bee first comes into

shot and the start of the first waggle run was defined as the start of the dance. The end of the dance was defined as the last circuit before the bee went out of shot. Foragers were classified into nectar foragers or pollen foragers. Pollen foragers are more likely to unload their pollen loads after performing waggle dances therefore they are easily recognisable with their pollen baskets on their hind legs (Visscher & Seeley, 1982; Calderone & Johnson, 2002). Although we did not test directly whether they were carrying water or nectar, a concurrent study conducted in the same area and in the same year showed that returning *A. cerana* foragers always carried nectar if they do not carry pollen (Ramesh et al. 2016).

The angle of the waggle run during the dance relative to gravity was determined at the start of each dance run using Photoshop's line tool. The dance angle codes the angle between the food source and the azimuth of the sun (von Frisch 1967). Whenever the image was not perfectly aligned in the vertical direction with the weighted line, I manually rotated it before measurement of the angle. I extracted the angles of the sun in the sky at the specific times of day at each of the hive locations from an online data source published and maintained by The Astronomical Applications Department of the U.S. Naval Observatory (2015) (http://aa.usno.navy.mil/data/docs/AltAz.php). To convert the angle danced by the bee to a compass angle for the food source relative to North, the danced angle was added if the bee was dancing right of vertical up to 180^{o} or subtracted if the bee was dancing left of vertical up to 180^{o} from the sun's azimuth.

The total duration of the dance was recorded and divided by the number of circuits the bee performed. One circuit is defined by a waggle run and the subsequent turning of the dancer before starting a new waggle run (Dyer &

Seeley 1991). It has been shown that the duration of one circuit as well as the duration of the waggle phase are both indicators of the distance to the food source encoded by the dancing bee (Michelsen et al. 1992). Here, I chose to base the analysis of the waggle dance durations on the methodology by Dyer & Seeley (1991) which recorded full circles that included the straight waggle run. Their experiments on Asian honeybee waggle dances have been conducted vigorously and represent the most extensive set of studies to date.

2.2.6 Distance calibration of the waggle dances

Variations of the encoded distance values have been reported for different populations and races of *A. mellifera* (Boch 1957; von Frisch 1967). There are also differences between the dances of *Apis* species (Lindauer 1957; Punchihewa et al. 1985; Dyer & Seeley 1991), however less is known about intraspecific variation between populations. Therefore, I conducted a calibration experiment that probed the distances indicated by dancing *A. cerana* in South India.

Individually marked bees were trained to a feeder offering a 30% sucrose solution in both the rice paddy and the rubber plantation (Fig. 12). The feeder was set up in progressive steps of 100, 150, 200 and 250m away from the hive. At each distance the feeder was in place for at least 1 hour and the time of visits by individually marked bees were recorded.

Figure 12. Feeder offering sucrose solution to honeybees. Photo by Katie Hall.

The duration of a dance performance was measured when a bee had been seen to leave a feeder shortly before (2 minutes max). This was done to ensure that the bee was dancing for the artificial feeder and not for another unknown food source. Up to 22 dances were decoded for each distance at which the feeder had been placed.

2.2.7 Statistical analysis

Linear statistics were conducted in IBM SPSS Statistics 23 for Windows. All data were checked for assumptions and normality using normal Q-Q plots and the Shapiro-Wilk test in IBM SPSS Statistics 23 for Windows. A chi-square goodness of fit test was conducted to test if there was a difference between the number of nectar dances compared to pollen dances at each location. A Mann-Whitney U test was performed to report if there was a difference between the distance flown for bees collecting either pollen or nectar in each location and across locations, respectively. To probe the similarity of data in the dance calibration experiments, linear regression lines were fitted in SPSS and then compared in Excel 2016 using the Real Statistics Function add in (Howell 2012). Bonferroni correction of the significance level was applied to all multiple comparison statistical tests.

Circular statistics were conducted in MATLAB R2017 for Windows using the toolbox CircStat (Berens 2009). A Rayleigh-U test was performed to test the significance of forage clustering in pollen and nectar foragers. A circular analogue to the Kruskal-Wallis test was performed to test if there was a significant difference between the mean angle flown from the nest of pollen and nectar foragers (Fisher 1995; Berens 2009). Both these tests were conducted on data from each habitat.

2.2.8 Ethical note

No licences or permits were required for this research. Permission from land owners and the local forestry department was given to place the hives on their land. The bees were not prevented from their normal behaviour and at the end of the experiments they were returned to the beekeeper.

2.3 Results

2.3.1 Calibration experiment

The distance to the food sources that is encoded in the bee dances shows variation between populations in the Western honeybee (Boch 1957; von Frisch 1967; Visscher & Seeley 1984). This could also be the case for the Eastern honeybee, as indicated by the differences described in the Dyer and Seeley and Lindauer studies which recorded the waggle dance durations for a feeder that was set at different distances (Lindauer 1957; Dyer & Seeley 1991). A calibration experiment was therefore conducted to establish the relationship between dance duration and encoded flight distance using an artificial feeder (Fig. 12). Figure 13 shows the function of the circuit duration relative to the feeder distance, which was compared to the dance dialect curves produced Lindauer (1956) and Dyer and Seeley (1991).

As the raw data from Lindauer (1956) and Dyer and Seeley (1991) was not available the regression lines were fitted to the reported mean values. The graph shows the regression lines for rice and rubber that have been fitted to the raw data set. However, for a comparison with the Lindauer (1956) and Dyer and Seeley (1991) lines, the regression lines were also fitted to the mean data points. There was no significant difference between any of the regression lines (Table 1.).

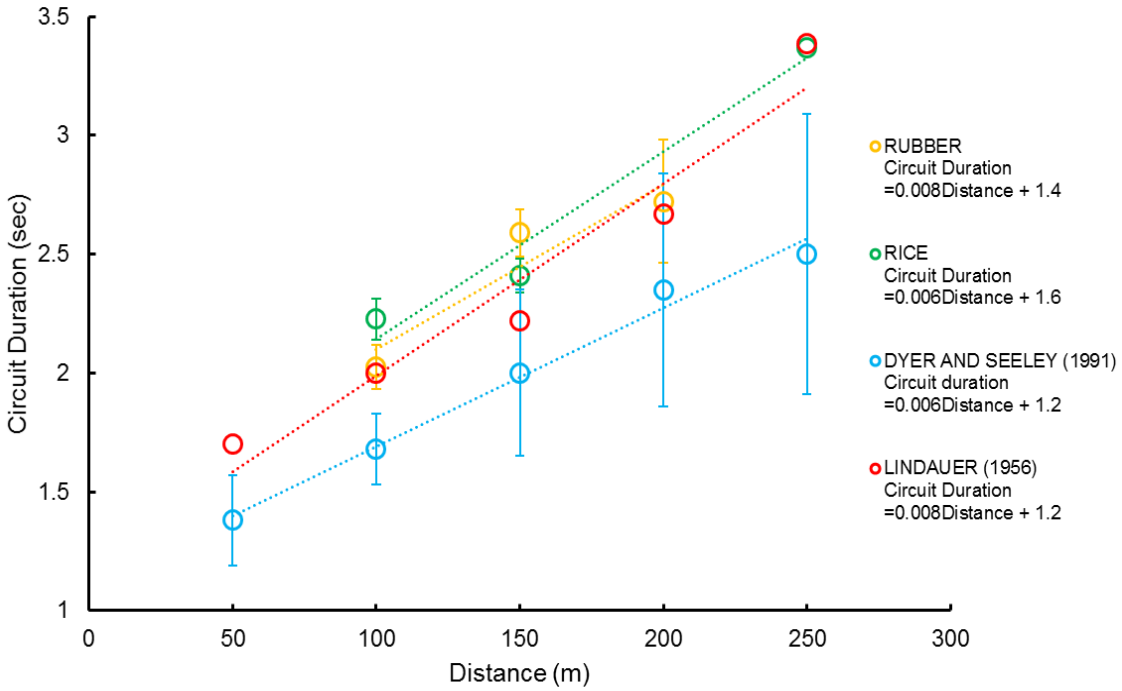

Figure 13. Distances encoded in the dances for feeder with 30% sucrose solution placed between 100m and 250m in the two locations. For comparison, the mean of the distances measured by Lindauer (1956) in Sri Lanka and the means and standard deviations from Dyer and Seeley for Apis cerana in a forest in Thailand (1991). Rubber: n=5 at 100m, n=9 at 150m and n=3 at 200m. Rice: n=22 at 100m, n=11 at 150m and n=1 at 250m. Dyer and Seeley (1991) n=13 at 50m, n=16 at 100m, n=22 at 150m, n=64 at 200m and n=24 at 250m. Lindauer (1956) did not display sample sizes. (Rubber: ($F(1,32)=9.466$, $p=.004$) with R^2 of .228) (Rice: ($F(1,15)=11.181$, $p=.004$) with R^2 of .427) (Dyer and Seeley(1991): ($F(1,3)=233.710$, $p<.001$) with R^2 of .987) (Lindauer (1956): ($F(1,3)=51.933$, $p=.006$) with R^2 of .945.

Comparison of regression slopes	Dyer and Seeley (1991)	Lindauer (1956)	Rubber	Rice
Dyer and Seeley (1991)				
Lindauer (1956)	t = **2.37** df = **6** p value = **0.055**			
Rubber	t = **1.29** df = **4** p = **0.266**	t = **0.23** df = **4** p = **0.830**		
Rice	t = **2.24** df = **4** p = **0.088**	t = **0.19** df = **4** p = **0.856**	t = **0.10** df = **2** p = **0.931**	

Table 1. Comparison of regression curves fitted to the means of the data due to lack of original data form Lindauer (1956) and Dyer and Seeley (1991).

2.3.2 Frequency of dances

In all four locations, there was significantly less pollen dances compared to nectar dances, this included decoded and undecoded dances (Rubber: X^2 (1) = 334.772, p<.001, Rice: X^2 (1) = 456.501, p<.001, City: X^2 (1) = 13.425, p<.001 and Forest edge: X^2 (1) = 434.562, p<.001) (Table 2).

Location	Total frequency of dances	Frequency of nectar dances	Nectar dances as % of total	Frequency of pollen dances	Pollen dances as % of total
Rubber plantation	913	833	91%	80	9%
Rice paddy	532	470	88%	62	62%
City	343	202	59%	141	41%
Forest edge	454	409	90%	45	10%

Table 2. Frequency of dances across the four locations

Total frequency plots, including undecoded and decoded dances, showed that the peak of activity of dances varied slightly between the different locations. In the rubber plantation frequency of dances peaked mid-morning between 8-10 am (Fig. 14) and 7-10am in the rice paddy (Fig. 15a). In the city the frequency of dances peaked later in the day, between 10-11am (Fig. 15b), whereas in the forest area this is again earlier, 9-10am (Fig. 15c), which is more similar to the rubber plantation.

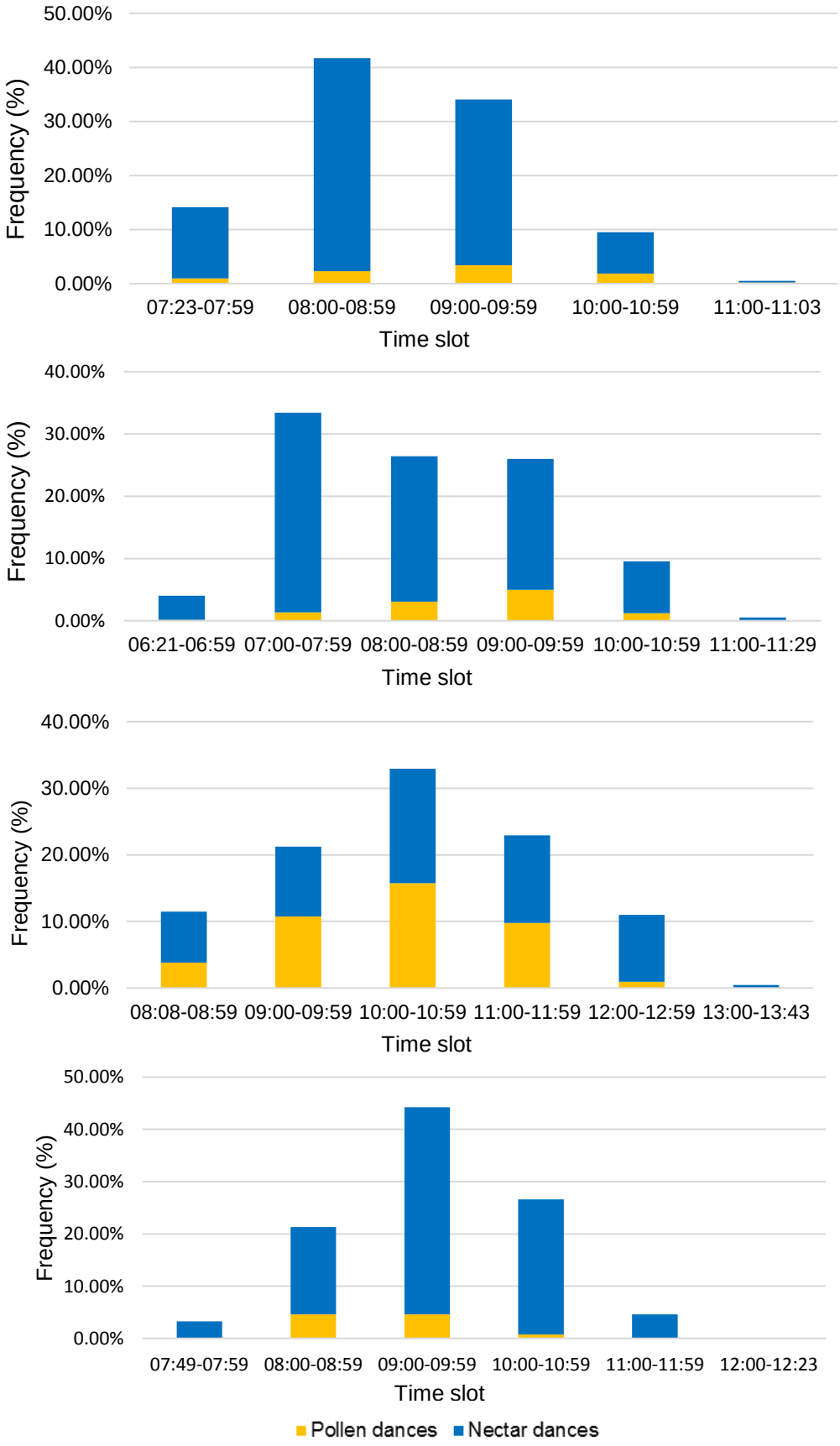

Figure 14. Total frequency of dances represented as a percentage during different time slots across the four habitats. (a) rubber plantation pooled over a period of 5 days in May, (b) rice paddy pooled over a period of 5 days in May/June 2016, (c) city pooled over a period of 5 days in May/June 2016 and (d) forest edge pooled over a period of 6 days in May 2016.

2.3.3 Spatial distribution of visited food sources

Analysis of the dances for pollen and nectar sources showed varied results of the direction and distances flown. This was seen both within and between the four locations.

2.3.3.1 Distance analysis

Across the four locations there is a clear peak in the frequency of dances that are advertising food sources at a distance of 200-400m from the hive (Fig. 16).

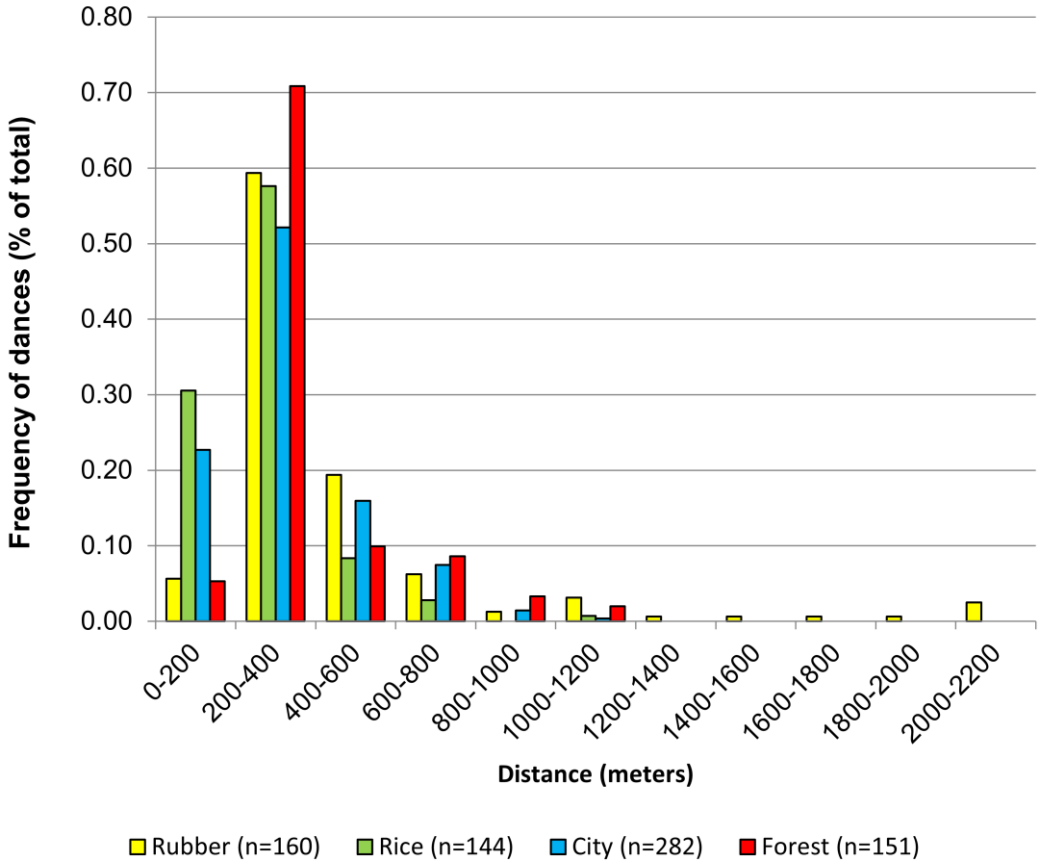

Figure 16. Normalised frequencies of distances indicated in waggle dances recorded in the four locations.

In three of the four locations, the rubber plantation, city and forest edge, there was a significant difference between the distance flown by bees collecting the different forage types. But, there was no preference to collect pollen or nectar at the further distances (Fig. 17, Fig. 18). In the rubber plantation and forest edge bees that were collecting nectar (Rubber Mdn=389m, Forest edge Mdn=334m) travelled significantly further than those collecting pollen (Rubber Mdn=326m, Forest edge Mdn=293m) However, in the city it was the other way around with pollen foragers (Mdn=348m) having to travel significantly further than nectar foragers (Mdn=239m)

In contrast, foragers in the rice paddy were collecting pollen and nectar from resources that were not significantly different distances away from the hive

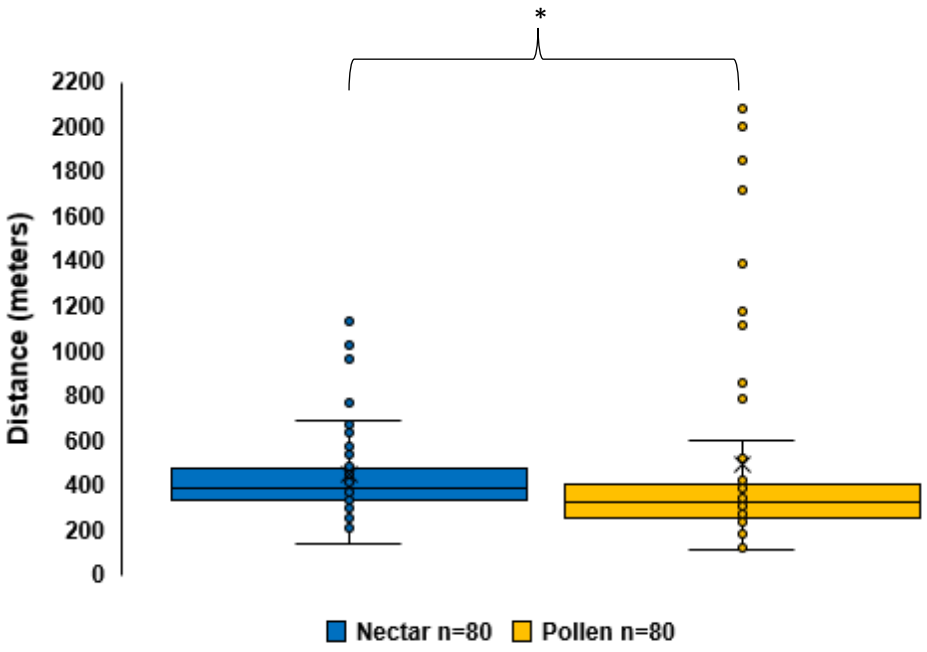

Figure 17. Distance decoded from waggle dances of bees foraging over a period of 5 days in May in the rubber plantation *U=2176, Z= -3.495, p< .001.

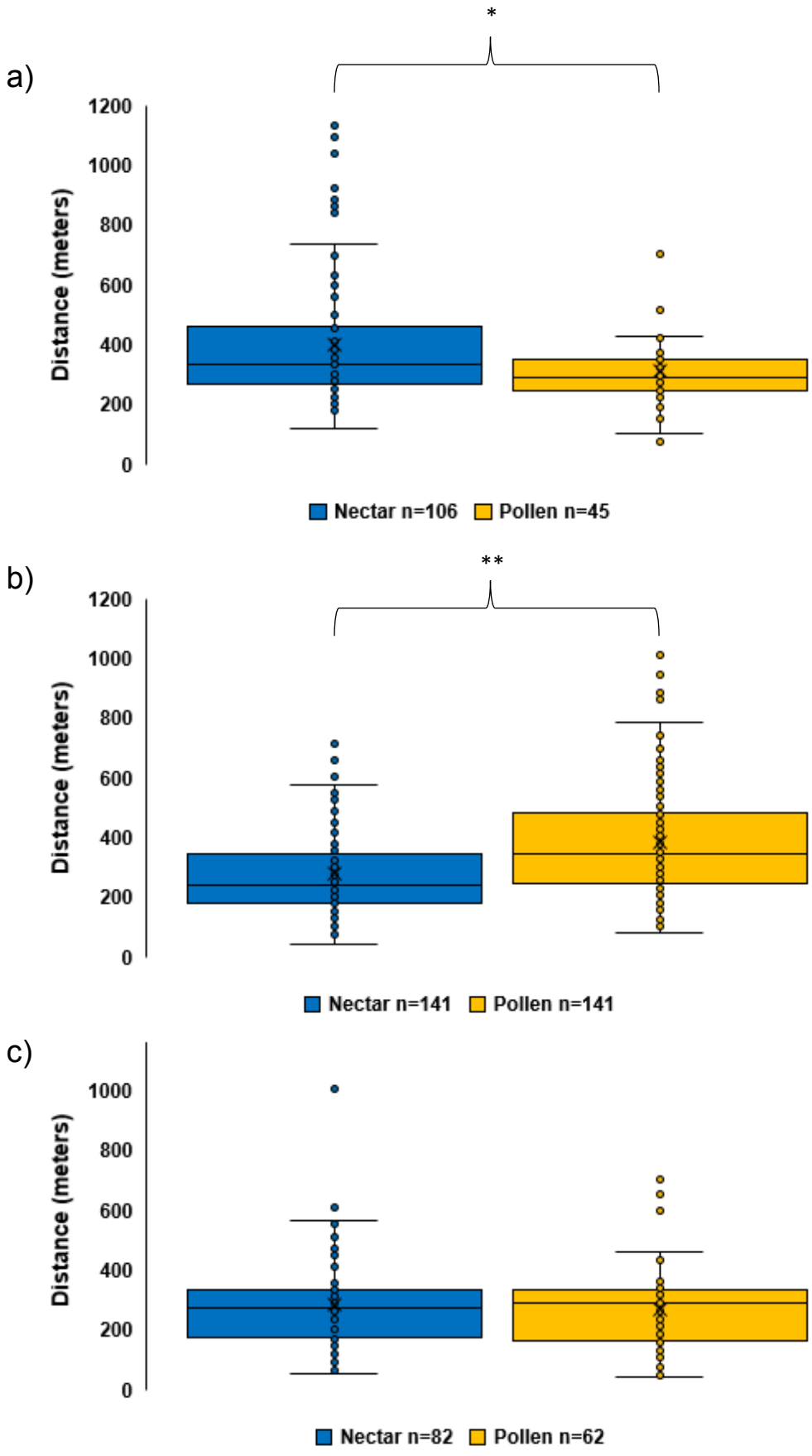

Figure 18. Distance decoded from waggle dances of bees foraging over a period of 6 days in May 2016 at the forest edge (a), in the city (b) for 5 days in May/June 2016 and 5 days in April/May 2016 in the rice paddy (c)
*U=1833.5, Z= -2.244, p= .025 **U=6283, Z= -5.342, p< .001.

Nectar foragers showed significant difference in their foraging distances across the four locations (Kruskal-Wallis test, H(3) = 82.189, p<.001) (Fig. 19). Nectar foragers in the rubber plantation had to travel significantly further compared to the other three locations Also, bees collecting nectar at the forest edge had to travel significantly further than bees collecting the same resource in the rice paddy (Fig. 19).

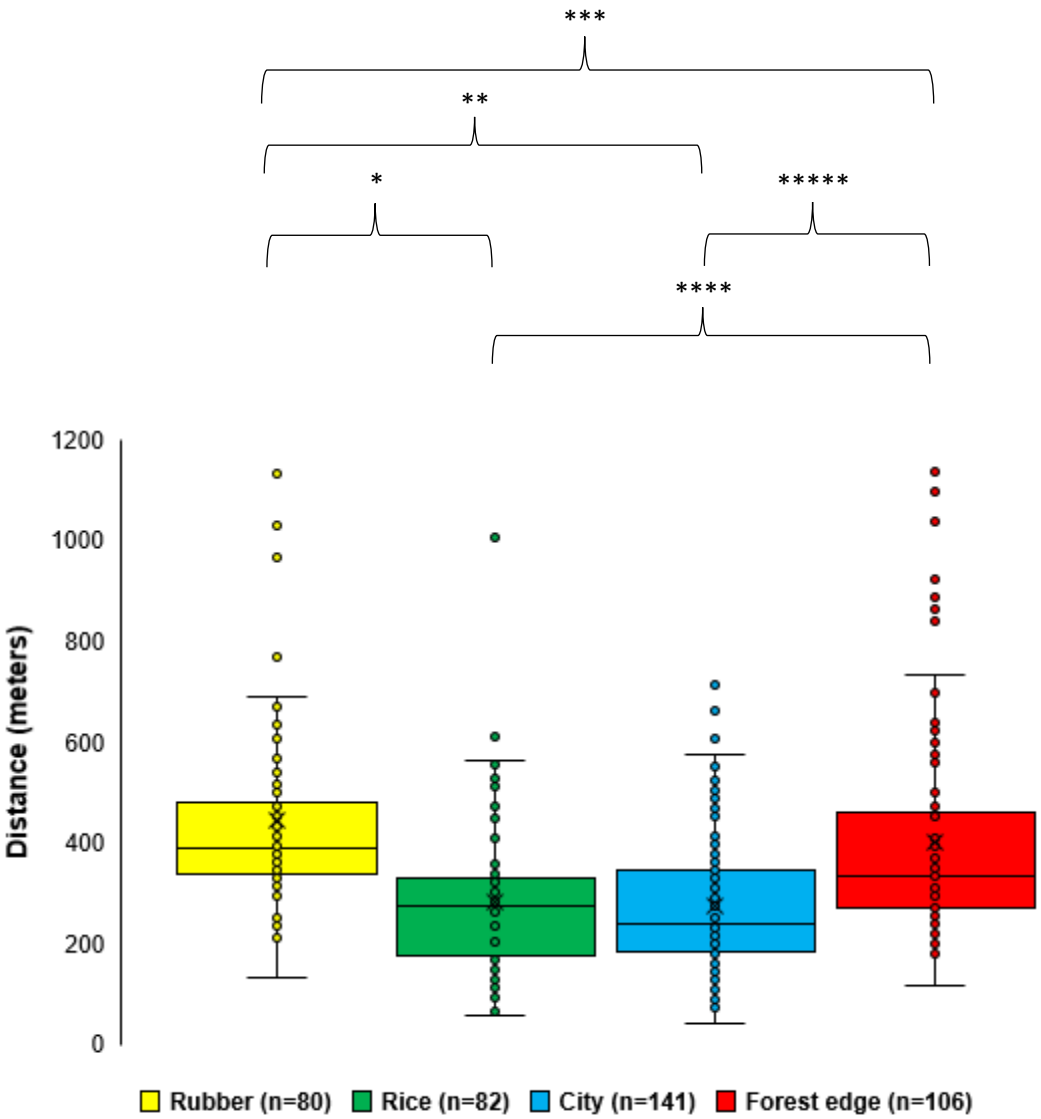

Figure 19. Comparison of distance decoded from waggle dances of nectar foraging bees across the four different locations. *(U=1223.5, z=-6.889, p<.001) **(U=2231, z=-7.463, p=<.001) ***(U=3056.5, z=-3.256, p=.001) ****(U=2706, z=-4.432, p<.001) *****(U=4324, z=-5.666, p<.001). Rubber Mdn= 389m, Rice Mdn=275m, City Mdn=239m and Forest edge Mdn= 334m.

Across the four locations there was a significant difference in the foraging distances of pollen foragers (Kruskal-Wallis test, H(3) = 21.083, p<.001) (Fig. 20). In the rubber plantation pollen foragers travelled significantly further than those collecting the same resource in the rice paddy . Also, bees collecting pollen had to travel significantly further in the city than those in the rice paddy (Fig. 20).

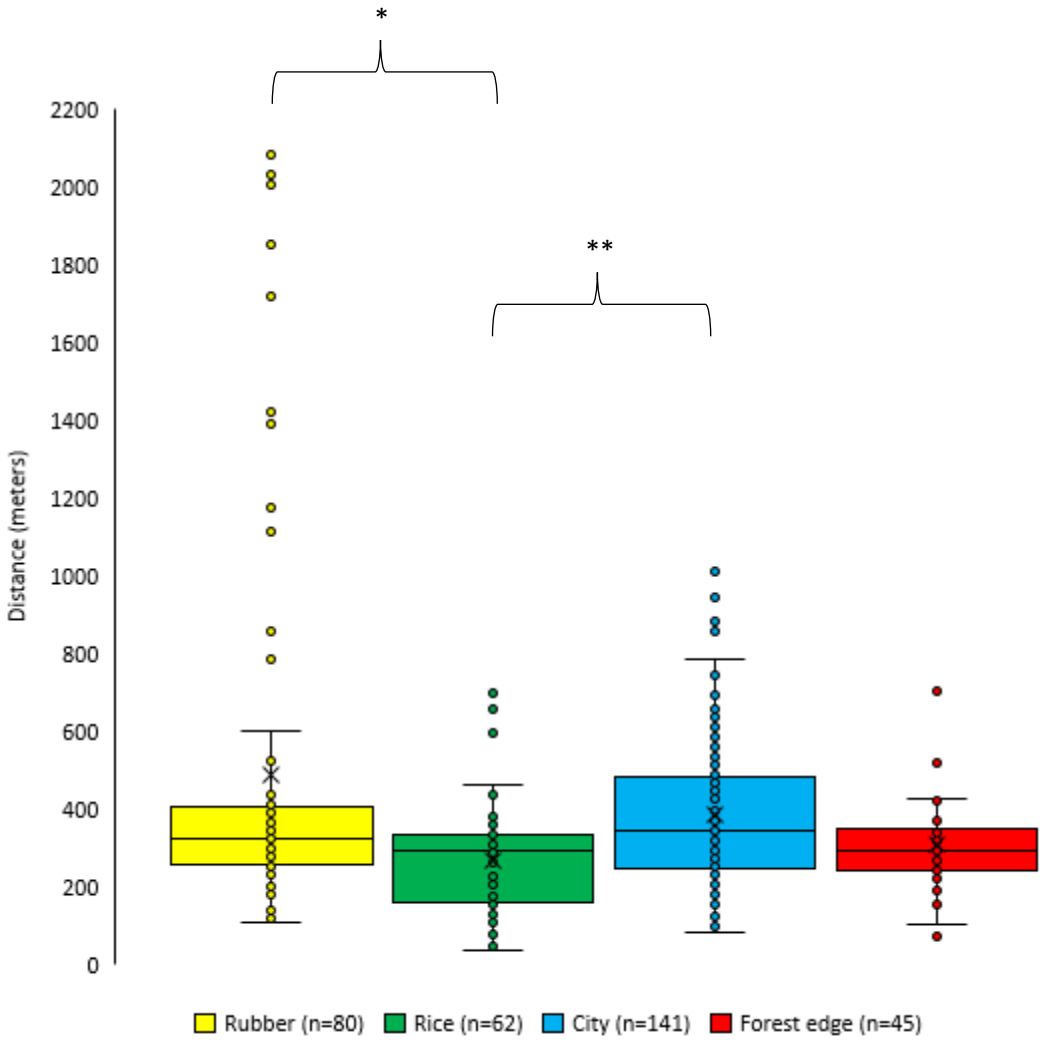

Figure 20. Comparison of distance decoded from waggle dances of pollen foraging bees across the four different locations. *(U=346, z=-4.340, p<.001) **(U=2742.5, z=-4.224, p<.001). Rubber Mdn= 326m, Rice Mdn=293m, City Mdn=348m and Forest edge Mdn= 293m.

2.3.3.2 Directional analysis

A directional analysis of the waggle dances was conducted both comparing the bees within and between foraging groups. Comparing within the foraging group allows for conclusions on whether the bees collecting the same resource foraged in the same direction. In contrast, comparing the directions of nectar and pollen foragers will determine whether the bees collecting different food sources flew in the same direction or not.

In the rubber plantation nectar foragers had significant directional clustering together which shows that they foraged at floral resources that were located in a similar direction (Mdn=12.30^{o} from N) (Rayleigh test, Z=14.480, p<.001). This was also apparent in the rice paddy (Mdn=281.44^{o} from N) (Rayleigh test, Z=11.554, p<.001) (Fig. 21). The pollen foragers in the rubber plantation also had significant directional clustering together (Mdn=96.87^{o} from N) (Rayleigh test, Z=3.5711, p=0.0276). However, in the rice paddy there was no significant directional clustering of pollen foragers (Mdn=99.07^{o} from N) (Rayleigh test, Z=2.146, p=0.1169) (Fig. 21). In contrast, pollen foragers in the city (Mdn=255.00^{o} from N) and at the forest edge (Mdn=13.49^{o} from N) had significant directional clustering in their respective locations (City: Rayleigh test, Z=35.429, p<.001) (Forest edge: Rayleigh test, Z=30.526, p<.001) (Fig. 22). The nectar foragers also showed directional clustering in the city (Mdn=244.53^{o} from N, Rayleigh test, Z=6.252, p=.0018) and at the forest edge (Mdn=18.07^{o} from N, Rayleigh test, Z=24.6162, p<.001) (Fig. 22).

There was no significant difference in the mean directions of nectar and pollen foragers in each location. This suggests that in their respective location nectar and pollen foragers were travelling in the same direction (rubber: p=.2699; rice: p=.5008; city: p=.4045; forest edge: p=.5573) (Fig 21a,b and Fig. 22a,b).

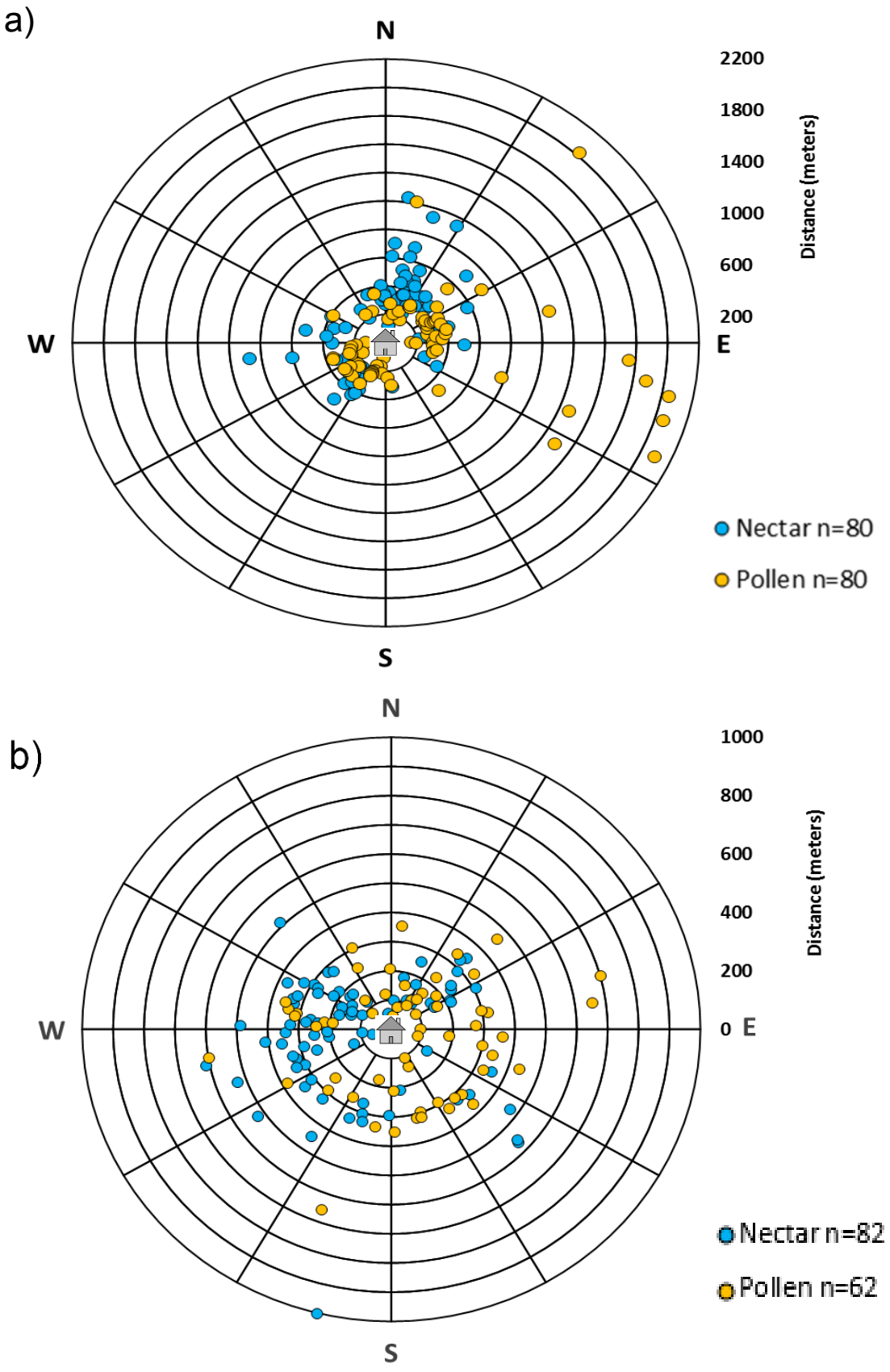

Figure 21. Compass plots showing the location of food sources decoded from waggle dances over period of period of 5 days in May 2016 in the rubber plantation (a) and 5 days in April/May 2016 in the rice paddy (b). The middle of the plot represents the hive and each line going out from the hive is 220m further away in (a) and 100m further away in (b).

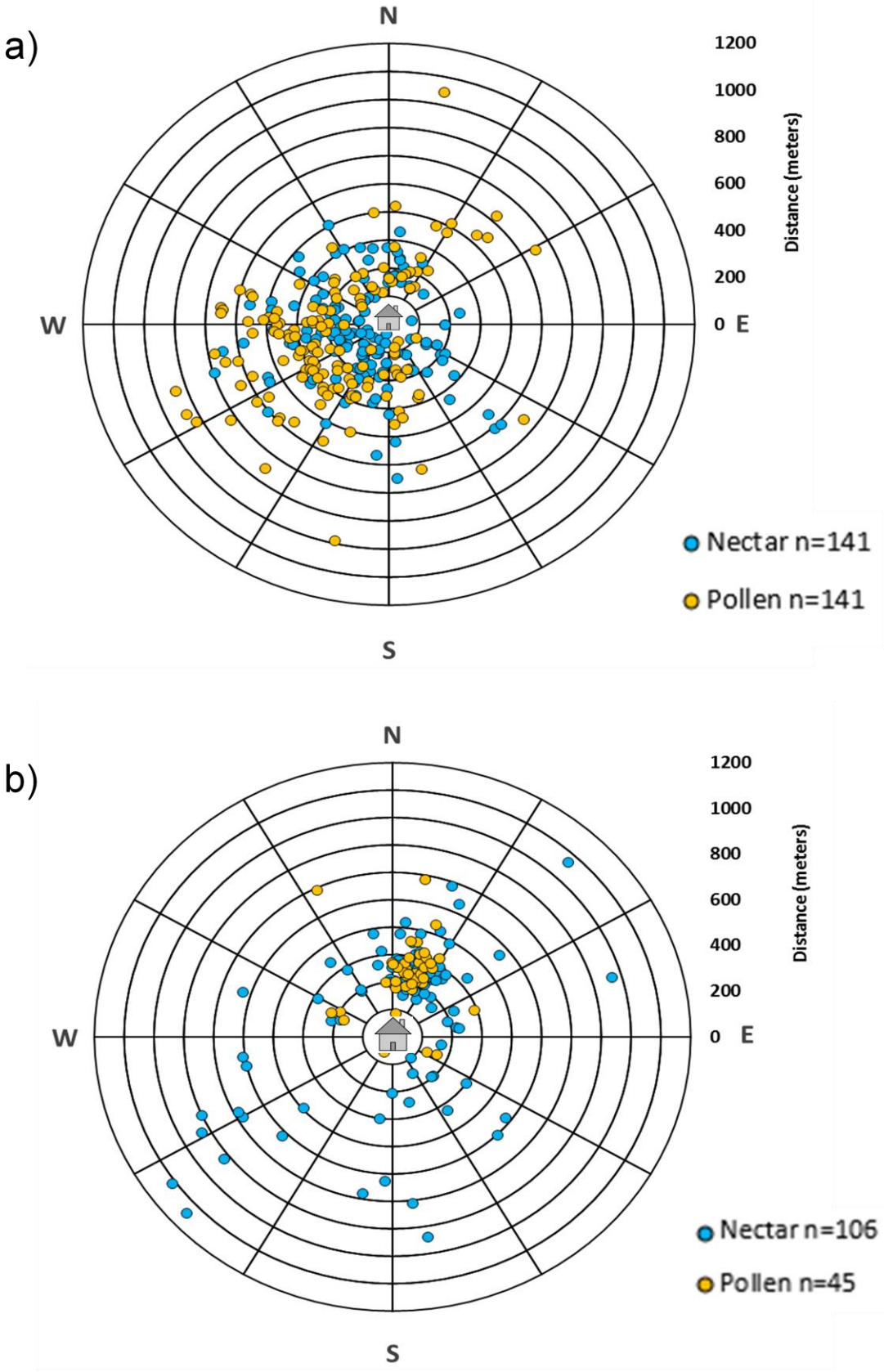

Figure 22. Compass plots showing the location of food sources decoded from waggle dances over period of period 5 days in May 2016/June in the city (a) and 6 days in May 2016 at the forest edge (b). The middle of the plot represents the hive and each line going out from the hive is 120m further away in both (a) and (b).

2.3.4 Profitability of the food sources visited

There was no significant difference in the number of waggle runs per dance across the four locations (Kruskal-Wallis test, H(3) = 6.318, p=.097) (Rubber: Mdn=8, Rice: Mdn=9, City: Mdn=9, Forest edge: Mdn=7) (Fig. 23). The amount of waggle runs per dance is a way for foragers to modulate and report on how profitable a food source is to follower bees. It has previously been reported that there is a positive linear relationship between a bee's dance response (waggle runs/dance) and the nectar-source profitability.

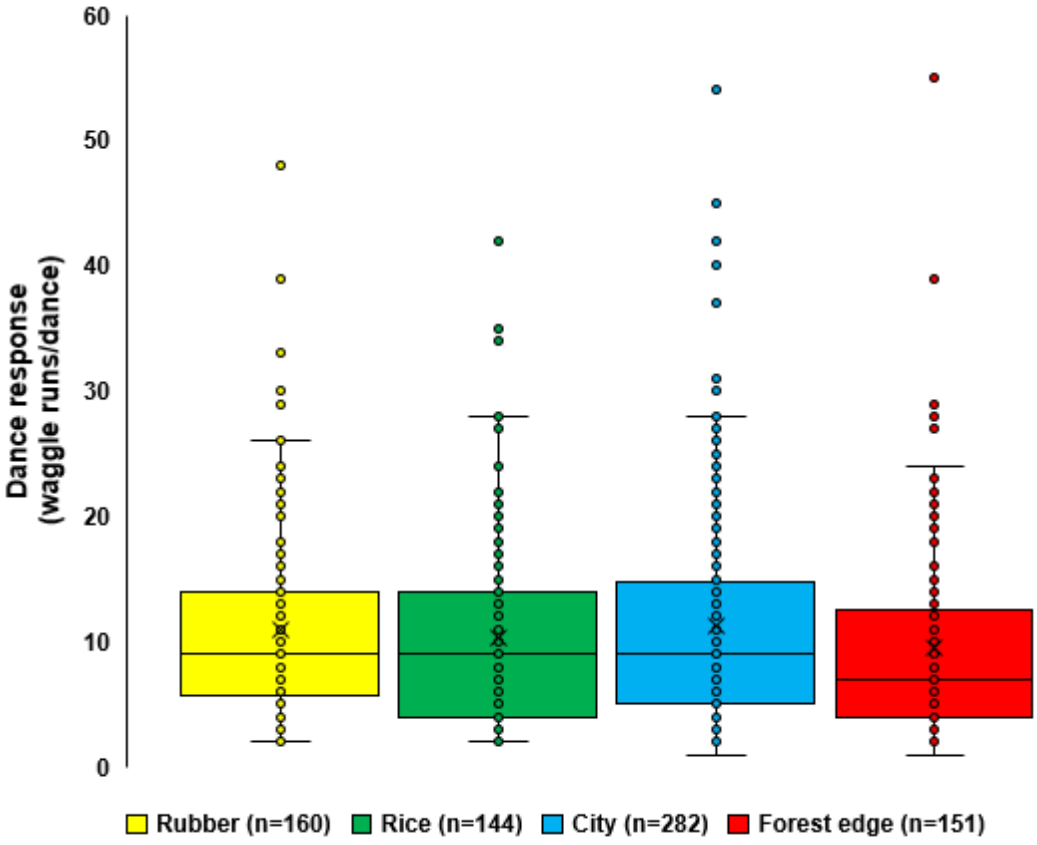

Figure 23. Comparison of dance response (waggle runs/dance) across the four locations

2.4 Discussion

Bee dances provide useful insights into the behaviour of foraging bees and how the available plant resources can shape and change foraging responses of a colony. Not only is it possible to look at the spatial distribution of foragers in different environments but you can also compare the responses to each major resource that foragers collect in different environments. This is because foraging *Apis cerana* bees returning with pollen do not seem to collect nectar, and those that return without pollen only carry nectar (Ramesh et al. 2016).

The analysis of 737 bee dances has shown that in all of the four locations there is a clear peak of waggle dances advertising food sources located 200-400m away from the hive (Fig. 16). Foraging distance was not a predictor of forage type, with pollen and nectar not consistently being collected at the same distances across the four locations (Fig. 17, 18). The bees in the rubber plantation had to collect food at significantly further distances than at the other three locations (Fig. 19, 20). Clustering of nectar foragers was seen in all four of the locations and this was also apparent in the pollen foragers except when the hive was located in the rice paddy (Fig. 21, 22). Profitability reported by the dances was not significant across the four habitats (Fig. 23). Profitability reported in the dances is not a direct report on how nectar rich food sources is, rather the bees integrates information about the forage site variables (distance from hive, sugar content, nectar abundance etc) and forage context variables (state of the colony, weather, time of day etc) (Seeley 1995). Therefore we can report that all the four locations had similar levels of profitable food sources.

These findings demonstrate that *Apis cerana* are flexible foragers and can adjust to various distributions of food sources in tropical human-impacted

landscapes of tropical South Asia. They forage over close and far distances and the ranges are within those reported earlier by Dyer & Seeley (1991) and Lindauer (1956). However, in contrast to those studies I rarely observed long distance foraging with only 5% of bees foraging beyond 716m. Dyer & Seeley (1991) reported that the top 5% of their foragers travelled further, with distances over 905m.

One important finding from my analysis is that the foraging ranges of *A. cerana* are much shorter than those reported for the Western honeybee *Apis mellifera* in similar human-modified landscapes (Steffan-Dewenter & Kuhn 2003). This could be due to the species-specific differences. For instances, median flight distances reported for *A. mellifera* is 1118.5m (Steffan-Dewenter & Kuhn 2003) and 1650m (Visscher & Seeley 1982). Whilst in a tropical forest, *A. cerana's* median flight distance was reported at 195m (Dyer & Seeley 1991). Here I found that in the human-modified landscapes of South India they flew over longer median distances (317m, see also Table 3).

Another intriguing finding was that in the rubber plantation bees had to fly further to find profitable food sources and therefore spend more effort and energy collecting food as compared to the other locations, where food seem to have been available at higher density more closely to the hive. Although *A. cerana* were evidently able to negotiate longer foraging distances, it could well be that foraging over longer distances might weaken hives when kept in those landscapes. Shortage of food resources has been attributed as a cause for high rates of absconding reported in farm beekeeping of *A. mellifera* (Schneider & McNally 1992). Lindauer (1956) observed that *A. cerana* colonies also usually abscond when the flowering depletes. One reason could be that *A. cerana* does not store as much food as *A. mellifera* and instead uses any surplus food to

feed more brood. It would be important to investigate the availability of floral resources in rubber plantation by mapping out plant abundancy and diversity, as beekeeping is mainly done by farmers in such settings of monoculture farms. This would help us to understand if this location is potentially sub-optimal for *A. cerana* in terms of food availability.

2.4.1 Dance dialect curves

It has been previously found that different populations of the Western honeybee, *A. mellifera,* show dialects in their dances. The distances indicated for artificial food sources were varied, and has been shown in a number of calibration experiments (e.g. Boch 1957; von Frisch 1967; Visscher & Seeley, 1982). Given the scarcity of such data for *A. cerana*, I could compare my calibration data only to those provided in two studies that were conducted in Sri Lanka (Lindauer 1956) and Thailand (Dyer & Seeley 1991).

Dyer & Seeley (1991) found that there was significant intraspecific dialect differences between populations of *A. cerana* in Thailand (Dyer & Seeley 1991) and Sri Lanka (Lindauer 1956). The dance dialect curves from Lindauer (1956) were steeper than that of Dyer & Seeley (1991). This would suggest regional dialect differences of *A. cerana* similar to what has previously been seen in races of *A. mellifera* (Boch 1957; Dyer & Seeley 1991). However, when comparing my results from India to that of Dyer & Seeley (1991) and Lindauer (1956) in Thailand and Sri Lanka respectively, I found no significant difference between the regression lines of the dance dialect curves (see Fig. 13 & Table. 1). This does not support the hypothesis presented by Dyer & Seeley (1991) that *Apis cerana* have regional dialect differences in tropical south Asia. However, there are a couple of factors that need to be considered. In my

experiments, due to logistics in the rice and rubber locations, feeders could only be placed a maximum of 250m away from the hive. In the experiments of Lindauer (1956) and Dyer & Seeley (1991) feeders were placed at maximum distances of 700m and 1200m respectively. Dyer & Seeley (1991) showed that when compared to the data from Lindauer (1956), there were significant differences between every flight distance except 150m, 200m and 300m and overall when fitting a linear regression line there were significant differences between the two data sets. This suggests that the differences between the dance dialects are greater at distances further away from the hive. This conclusion by Dyer & Seeley (1991) shouldn't affect my results because the main foraging ranges in my study are at shorter distances and over these distances there was no significant difference between the rubber and rice curves compared to Dyer & Seeley (1991). Dyer & Seeley (1991) recorded waggle dances from feeders placed furthest from the hive and they also had a larger sample size than Lindauer (1956) and myself. Therefore I used the dance dialect curve of Dyer & Seeley (1991) to estimate the distances flown from the observed waggle dances.

I conducted the calibration experiment in two locations, one more cluttered environment with many tress and understorey shrubs (rubber plantation) and another more open, a rice paddy surrounded by houses with gardens, small fields and plantations. As optic flow helps bees to gauge distance (Srinivasan et al. 1997; Srinivasan et al. 2000; Esch 2001; Si et al. 2003), it has been suggest that differing density of features in the landscapes may affect the rate of optic flow perceived by the flying bees which could then misestimate the real distance flown.

An experiment by Heran (1956) involved placing feeders at that same distance but either uphill or downhill from the hive. Honeybees travelling to the feeder uphill elicited waggle dances that were longer than the subsequent dances from a feeder placed downhill from the hive (Heran 1956; Srinivasan 2014). Lab experiments have shown that when a honey bee flies inside a tunnel that has been lined with horizontal stripes it overestimates the distance flown compared to if no stripes were present. In the lined condition the optic flow experienced by the bee is higher than the no stripes condition (Srinivasan et al. 2000).

Based on these findings, Tautz et al. (2004) hypothesised that varying visual cues of the landscape can lead to variations in distance estimation. They trained bees to a feeder along two different routes. One route was over land whilst the other was over land at first, then water and then land again. They found that when the bees flew over water they produced a significantly flatter slope of the waggle-duration versus distance regression curve, compared to the curve they produced when they flew over land. This shows that the bee's odometer does not run at a constant rate and is dependent on the terrain that the bee flies across. So far this seems to be the only evidence supporting this hypothesis. Note that the variation in those experiment required bees to negotiate a lake, and so far they are known to avoid navigationally challenging options such as mountains (von Frisch 1967). Flying over open water provides very little optic flow and it can be harder for bees to control their flight. In view of lacking evidence for terrestrial habitats, it should be assumed that dialects are more likely to be caused by population differences rather than environmental features.

However, I considered the possibility that distance estimation might differ depending on whether the hive was located in the cluttered plantation or the

open rice paddy. The data did not differ however. Even though to the human observer the rice paddy was a very open terrain with a different ground pattern from the rubber plantation (see Fig. 9) which was cluttered with vegetation and branches, it seems that the two locations both provide similar optic flow cues to the bees and do not impact on their distance estimation.

Another point that deserves some reflection is the fact that I used foragers from the same colony for both calibration experiments. It has been shown that genetic variation can influence the dance dialect of honeybees (Rinderer & Beaman 1995; Johnson et al. 2002). Therefore, I am not able to conclude that the similarity of distance estimation in the waggle dances results from similarity in optic flow cues in both locations, and more research with unrelated hives would be required to weaken the impact of potential genetic effects.

2.4.2 Dancing for nectar and pollen locations

In all four locations, the Eastern honeybee (*Apis cerana*) had significantly more nectar foragers than pollen foragers performing waggle dances. Previous longitudinal studies with *Apis cerana* have found that throughout the year there are always more nectar foragers than pollen forages, with the difference widening during the rainy season (Reddy 1980; Mattu & Verma 1985; Ramesh et al. 2016). During the wet season *A. cerana* are able to go out between rains as it is warm and plant resources are available. They just do not forage as regularly and seem to rely more on stocked food (Reddy 1980). The fluctuations in the proportion of forage type collected may be due to the differences of availability of pollen and nectar from plants during the different seasons (Mattu & Verma 1985). Due to the low availability of pollen during the wet season it has been reported that *A. cerana* stop producing brood all together (Woyke 1980).

Thus, the wet season seems to pose some challenges to the colony cycle that are comparable to the winter in the temperate regions when *A. mellifera* colonies stop breeding and rely on food stores (Simpson 1961).

Similar to *A. cerana*, it has been reported that over two years of observations of dances of the Western honeybee (*Apis mellifera mellifera*) in the UK, there were fewer pollen dances than nectar dances, ranging between 2-41% each month of the total number of dances (Couvillon et al. 2015). But this does not seem to apply to all races and populations of the Western honeybee. A study on African honeybees (*Apis mellifera scutellata*) found that these bees have a much higher proportion of pollen dances, with 60% of the total dances recruiting to pollen sites (Schneider 1989). The differences in foraging priority between the two subspecies of the Western honeybee has been attributed to the different ecological landscapes and the impact it has on the life-cycle of the honeybees (Schneider 1989; Couvillon et al. 2015). African honeybees (*A.m.scutellata*) live predominantly in tropical habitats and have an extended foraging season, higher predation risks and high swarming rates as compared to temperate Western honeybees. Therefore, to maintain the population level it appears that they devote more energy into brood rearing and require a large amount of pollen (Schneider 1989). The temperate Western honeybee (*A.m.mellifera*) on the other hand, must build a large store of honey to survive during hibernation in winter and they therefore prioritise nectar foraging (Schneider 1989; Couvillon et al. 2015). In the present study, the Eastern honeybees have shown a priority for nectar foraging over pollen foraging unlike the tropical African honeybee, although they live in similar climates, and more like the temperate Western honeybee. It could well be that an extended pause in the breeding cycle dictates the brood levels and thus the need for pollen stores during the

flowering season. More research should scrutinise these findings to understand how macroclimatic conditions, but also the adaptations in breeding cycle, colony development, colony size and possible migration shape the adaptive allocation of nectar and pollen foraging in the different honeybee species.

2.4.3 Foraging ranges of honeybees

Bees can travel over impressively long distances to find profitable food sources. For instance, the Western honeybee has shown maximal foraging distances of 10.9km (Visscher & Seeley 1982). Although bees are small in body size, they are able to navigate and successfully return. But given that longer distances pose more risks of being predated or get lost through navigational errors or wind drift, experience damage or starvation, how relevant is this ability for their everyday foraging decisions? In other words, do they prefer to stay closer to the hive when they can? It is known that the profitability indicated in the waggle dances by Western honeybees declines with further distance (von Frisch 1967), which implies that foragers should prefer food sources that are closer to the hive. However, it is also known that honeybees and bumblebees do not forage in the vicinity of a colony (Cresswell et al. 2000; Chapman et al. 2003). To test this idea, one has to scrutinise the bees' usual foraging distances, and the waggle dance gives an easy insight into those.

Intraspecific comparisons of the data collected in the present study with those reported in the literature shows that there are similarities in the foraging ranges of *A. cerana* across regions in Asia (Table. 3). Dyer & Seeley (1991) and Punchihewa et al (1985) have previously reported foraging distances recorded in waggle dances. They found that *A. cerana* prefer to forage within 400m of their hive. The analysis of dances in the present study, combining nectar and

pollen foragers, showed that 50% of dances signalled the location of food sources at a median distance of 317m from the hive. There is also a clear peak in dance frequency across all of the four locations advertising food sources that were 200-400m away from the hive (Fig. 16).

Comparison of foraging ranges within the Asian honeybees give different results. Punchihewa et al (1985) compared the waggle dances of three of the *Apis* species; *A. cerana, A. dorsata* (the giant honeybee) and *A. florea* which is much smaller than *A. cerana*. Although their results suggested that the giant honeybees, *A. dorsata,* easily forages over long distances, all three of the Apis species seemed to prefer to forage within 400m of the nest. In contrast, Dyer & Seeley (1991) report significant differences in the flight ranges across the three Asian honeybee species. *A. dorsata* had the longest flight ranges with 50% of its foragers flying up nearly 11km away from the hive, whilst *A. florea* and *A. cerana* had significantly shorter foraging distances with 50% of their foragers flying up to 268m and 195m away from the hive respectively.

The differences in common flight distances resembled those in maximum flight ranges from natural feeding sites measured by Dyer & Seeley (1991). The giant honeybees, *A. dorsata,* flew up to a maximum distance of 22km. The smaller-sized *A. florea* was observed dancing for a location at a distance of 15.2km and the smallest maximal distance was held by *A. cerana* at 2.5km maximum distance flown.

Differences between the foraging ranges in the Asian honeybees has previously been attributed to the different body sizes of the bees. *A. dorsata* was believed to travel further due to being the largest of the Asian honeybees (Lindauer 1957). A meta-analysis study analysed the data collected for 62 bee species

and found a positive correlation between body size and flight distances (Greenleaf et al. 2007). Whilst the published data reported for the differently-sized Asian honeybees above (Punchihewa et al. 1985; Dyer & Seeley 1991), do not fully conform with these findings, some new findings do. Comparing the responses to an artificial feeder located at various distances to the hive, Nevard et al. (2016) found that *A. cerana* and the very small stingless bee *Tetragonula (Trigona) irridipennis* followed the feeder over different distances. The stingless bees gave up much earlier, whilst *A. cerana* did not seem to show much hesitation to visit the feeder when offered at distances up to 200m. However, *A. cerana* from India, as reported in the present study, seem to have shorter flight ranges than the smaller-sized bee *A. florea* from Thailand (Dyer & Seeley 1991). More work needs to be done in Asian bees to understand how body size may determine foraging ranges in tropical bees.

The difference in body size between *A. cerana* and *A. mellifera* is not as large as what has been seen between *A. cerana* and *T. irridipennis*. However the comparison of flight ranges between the Eastern and Western honeybee are quite conclusive in saying that the Eastern honeybee has significantly shorter foraging ranges (Lindauer 1956; Dyer & Seeley 1991) (Table. 3).

In a temperate forest in North America it was observed by Visscher & Seeley (1982) that 50% of the foraging of *A. mellifera* occurred within 1650m of the hive and the maximum distance danced was 10.9km. Shorter foraging distances was reported by Waddington et al (1994) of *A. mellifera* in suburban environments, where 50% of foraging occurred 745-935m across the different colonies. Observations in structural landscapes impacted by humans in mainland Europe show foraging ranges that fit in-between the results of Waddington et al (1994)

and Visscher & Seeley (1982). 50% of the foraging occurred within 1181.5m, the overall mean distance was 1526m and maximum foraging distances are at ~6km from the hive (Steffan-Dewenter & Kuhn 2003).

	Natural foraging		
	Distances of which a percentage of dances have occurred		Max distance danced (km)
	50% (meters)	95% (meters)	
A. mellifera			
North America	1650	6000	10.9
Germany	1118.5	-	~6.0
A. cerana			
Thailand	195	905	2.5
India (Fig. 16)	316	716	2.1
A. florea			
Thailand	268	1323	15.2
A. dorsata			
Thailand	863	3810	21.8

Table 3. Foraging ranges of different species of honeybee observed from decoded waggle dances for natural food sources. Data from North America (Visscher and Seeley 1982), Germany (Steffan-Dewenter & Kuhn 2003) and Thailand (Dyer and Seeley 1991).

It appears that there are interspecific differences in the flight ranges of species of honeybee. Although more work needs to be done to understand the intraspecific differences amongst the Asian honeybees, the comparison of *A. cerana* and the Western honeybee *A. mellifera* suggests a clear difference. *A. cerana* seem to prefer foraging over much shorter distance ranges.

It has been suggested in the literature that the intraspecific differences seen amongst populations and races of the Western honeybee are caused by landscape structures, dominant habitat types and the distribution of high-quality food sources that can vary substantially in different habitats (Waddington et al. 1994; Steffan-Dewenter & Kuhn 2003). This notion is further supported by a report finding that in an area of highly dense flower availability in agricultural land *A. mellifera* was observed to only have median foraging ranges of a few hundred meters (Free 1970; Visscher & Seeley 1982). It has also been suggested that flight distance may be impacted by the size of the colony (Waddington et al. 1994). Larger colonies have increased numbers of workers compared to smaller colonies and therefore there is a higher amount of inter-colony competition and food resources closer to the hive deplete quicker (Michener 1974). These explanations for intraspecific differences in *A. mellifera* can also be applied to explain the differences in interspecific differences between *A. mellifera* and *A. cerana*. From a concurrent study in the same locations as my study, we know that the majority of pollen collected by *A. cerana* is coconut pollen (Nevard et al. 2016). This resource was readily available in all of the four locations and therefore was not the restricting factor causing the preference for foraging within 200-400m of the nest seen in figure 16. Also as some of the bees did indicate going up to 2.1km from the nest we know that the ability to travel further was also not a limiting factor. Therefore foraging efficiency must have been the driving force for the preference by *A. cerana* to focus on food sources located 200-400m from the hive. When comparing this foraging preference to the *A. mellifera* studies we see that *A. mellifera* travel significantly further than *A. cerana* (Lindauer 1956; Dyer & Seeley 1991). Currently experiments comparing the foraging ranges of *A.*

mellifera and *A. cerana* have not been completed in the same environment. *A. mellifera* has been introduced to India however they are not as well adapted to the hot tropical climate compared to *A. cerana* (Mishra and Kumar 1997). Therefore it is difficult to account for changes of behaviour and physiology due to a lack of acclimation or potentially limitations in their ability to adjust to the 'wrong' climate. Nevertheless, such an experiment could be of interest to find out if the introduction of the Western honeybee could cause competition for food sources between itself and its Eastern sister species due to similar foraging ranges in these tropical habitats.

2.4.4 Foraging comparison

The Eastern honeybee showed flexibility and the ability to forage in the different locations which all showed strong anthropogenic influences, such as agricultural plantations and fields around the forest edge. Remarkably, bees exploited food sources both in fields and the forest as the distribution of foraging locations suggests. However, there were also some interesting differences in the spatial aspects of foraging both within and between habitats of each location.

2.4.4.1 Foraging distances negotiated by nectar and pollen foragers

At three sites I found differences between the foraging ranges of pollen and nectar foragers, however they were diametrically opposed. Whilst in the city pollen foragers travelled on average 109m further than nectar foragers, it was the other way around in the rubber plantation and at the forest edge. In those two locations nectar foragers travelled further on average 63m in the rubber plantation and 41m at the forest edge (Fig. 17, 18). Taken together with the finding that in the rice paddy there was no difference in foraging ranges, it appears that foraging distances are not predicted by the type of resource that

the bees would collect. This is in line with findings for the Western honeybee studied in North America. In Florida there was no significant difference in the foraging distances of pollen and nectar foragers. However in California nectar foragers had to travel on average 192m further than pollen foragers (Waddington et al. 1994). One limitation of my study was that dances were recorded once for 5 days in each location. It would be worthwhile to repeat the recordings at various time points across the flowering season, to understand whether foraging distances might vary between pollen and nectar foragers.

If one type of food resource is constantly exploited at significantly further distances compared to the other it suggests that pollen and nectar is not being collected at the same flower patches, or there is higher competition for one of the resources. A study by Couvillon et al. (2015) was conducted over 2 years and it found that for some months pollen was collected at farther distances, but in other months nectar was foraged at further distances from the hive. For example during the summer nectar competition is at its highest so bees collecting this resource must travel further (Couvillon et al. (2014). Pollen however is least likely to be collected as there is less brood rearing. This suggests that foraging ranges of *A. mellifera* change across the flowering season being influenced by the condition of the hive and floral resources available in the environment (Couvillon et al. 2015).

Fluctuations in the foraging activity across a yearly cycle has been seen in *A. cerana* and it shows similar patterns of fluctuations, compared to *A. mellifera*, in the requirement of a specific food resource during the year (Reddy 1980; Mattu & Verma 1985). However a longitudinal study looking at how foraging ranges of pollen and nectar foragers fluctuate throughout the year has yet to be completed. Further experiments should focus on a prolonged experiment on *A*.

cerana to see if the observation in *A. mellifera* are true of the Eastern species as well. This would also help to conclude whether any of the habitats are impaired in offering sufficient foraging resources to sustain wild and managed *A. cerana* populations.

When separating foraging ranges by forage type there were significant differences across and between the four locations. Overall there were more significant differences between the rubber plantation and the other locations. I found that nectar foragers in the rubber plantation travelled significantly further as compared to the other three locations (Fig. 19). Also pollen foragers in the rubber plantation had to travel further than those in the rice paddy (Fig. 20). This suggests that despite the dense vegetation offering nectar and pollen close to the hive, these resources were limited in availability. Most likely there was higher competition for these food resources compared to the other locations causing the bees to travel further.

Not many studies have yet scrutinised the relationship between landscape features, food availability and foraging responses in bees, less so in other pollinating insects. The general approach is to look at abundance rather than foraging behaviour (Biesmeijer et al. 2006; Potts et al. 2010). A first important study that addressed foraging behaviour in different natural landscapes in Germany was done by Steffan-Dewenter & Kuhn (2003). They found that there were differences between foraging distances of *A*. *mellifera* in simple and complex landscapes, however they were not the same when considering type of forage. The mean foraging distances of nectar foragers did not differ significantly between simple and complex landscapes. Conversely, pollen foragers had to travel further in simple as compared to complex landscapes. This difference was more pronounced in June and less so in May and July. This

was because in May and July mass-flowering resources such as oilseed rape and apple trees were in full bloom. Simple landscapes in this experiment were dominated by annual crops, which led to increased competition during June when the crops were not providing floral resources (Steffan-Dewenter & Kuhn 2003). To have a better understanding of how the landscape effects the foraging behaviour of *A*. *cerana*, a wide scale study using GIS and studying waggle dances of *A. cerana* in different structural landscapes should be undertaken. This would enable us to determine the effects large-scale distribution of foraging resources might have on foraging behaviour of Eastern honeybee. This seems to be a timely question, when considering that humans are impacting and changing the structural landscapes in Asia by increasing agriculture and fragmentation of natural forests. We need to know how *A. cerana* will respond to such alterations in the landscape as it is an important pollinator for wild and domesticated plants (Partap & Verma 1994; Partap et al. 2000; Joshi & Joshi 2010). Especially as the present study demonstrates that knowledge obtained for closely related sister species, such as the Western honeybees, cannot be simply extrapolated to this species.

In my study there was also a significant difference between the distances flown by pollen foragers in the city and the rice paddy, suggesting that pollen resources might have been distributed more scarcely. However, this difference is very small and must be therefore considered carefully. There could be a difference due to the positioning of the hives. In the city the hives were housed on top of a 3-storey building. Experiments by Esch & Burns (1996) have concluded that higher flight altitudes can cause underestimation of distance to a food source. Foragers visiting a balloon feeder underestimated the distance flown to the feeder when it was raised from ground level to 90m (Esch & Burns

1995). Also bees that were housed on a high building signalled a much shorter distance to a food source on another high building than the actual flight distance. Estimation of distance flown is determined by optic flow and the closer to the ground the bee flies the faster and further images move across the retina (Esch & Burns 1996). Therefore at higher altitudes optic flow is less. It is likely that the bees in the city underestimated the distance flown and danced somewhat shorter distances than flown in reality (Esch & Burns 1996). Although not tested yet, it is likely that the mechanism in *A. cerana* for distance estimation is similar to *A. mellifera*. A calibration experiment that incorporates height, similar to those conducted by Esch & Burns (1996), would answer this question.

2.3.4.2 Comparison of flight directions in foraging honeybees

Even though the comparison between foraging distances of pollen and nectar foragers has been examined previously (Schneieder 1989; Waddington et al. 1994; Couvillon et al. 2015), there is a distinct lack of comparisons of foraging directions. In the present study there were no significant differences between the direction in which pollen and nectar foragers travelled in any of the locations (Fig. 20, 21). This would suggest that foraging patches of nectar and pollen were available in the same direction and possibly plants offered rewards of both pollen and nectar to the different forager types. In each of the four locations the foraging directions clustered for nectar foragers which suggests that nectar foragers had a small distribution among resource patches and were generally foraging in the same direction as each other. For pollen foragers clustering of feeding was significant in the rubber plantation, city and forest edge. Clustering of foraging patches has been seen in studying *A. mellifera mellifera* in temperate forests in the US (Visscher & Seeley 1982) and also when studying

the African honeybee, *A. mellifera scutellata,* in Botswana (Schneider 1989). Clustering was significant for both forage types but pollen foragers showed increasingly concentration and adjustment to foraging patches as some diminished and others became rewarding (Visscher & Seeley 1982; Schneider 1989). However Waddington et al (1994) investigating the distribution of *A. mellifera mellifera* foragers in Florida and California did not see a strong concentration of foraging on small patches by nectar or pollen foragers. They found that waggle dances indicated floral resources that were widely scattered and this was a good representation of the actual distribution of food sources in residential gardens and flowering trees in the environment.

Thus it seems that bees handle different distributions of foraging resources easily and with flexibility. Research does suggest however that the distribution of forage could affect the evaluation of profitability that is communicated by the dancers of the Western honeybee, *A. mellifera.* This could affect the distribution of foragers in the landscape attracting recruits to the most profitable area in the landscape (Waddington et al. 1998; Seeley et al. 2000; Nicholls & Hempel de Ibarra 2017). Therefore clustering of waggle dances seen in this study by *A. cerana* could reflect similar preferences for locations, with dense clusters of food resources not a good representation of the overall distribution of floral rewards in the landscape. It could well be that Eastern honeybee have high selectivity for dense rewarding patches which in turn can influence the threshold for triggering dances within a range. Whilst I found that profitability signalled in the dances did not differ between sites, as one would expect (von Frisch 1967; Seeley et al. 2000), the question of the dance threshold and regulation of reward evaluation is yet to be studied in Eastern honeybees.

Chapter 3. Outlook

This study has addressed for the first time how *Apis cerana* bees distribute their foragers in the tropical environment of South Asia in the context of human transformation of landscape due to agricultural activity and forest fragmentation. It helps to improve our understanding of the behaviour and ecology of the Eastern honeybee, *A. cerana*, which so far has been little studied, despite being a major source of income for farmers from honey production and being managed widely across India and South Asia.

Further research should be focussed on the foraging behaviour of the Eastern honeybee in the rubber plantations. The rubber plantations are normally where beekeepers in Kerala, South India, place managed hives of the Eastern honeybee. However, the preliminary results from this study indicate that these plantations might be sub-optimal in terms of density and distribution of food resources, at least in the later part of the dry and flowering season. This is not considering aspects of nutrition, where bees might not receive the necessary breadth of nutrients to maintain healthy and resistant colonies (Nicolson 2011). Therefore, it might be worthwhile to investigate more closely the nutritional requirements and provision of food in agricultural areas for *Apis cerana*. It might well be more beneficial for the beekeepers to move the hives closer to areas with more diverse food sources as open areas lined with gardens and small fields, or periurban areas of cities and towns to allow bees to exploit food sources that are closer to the hive. This could help to obtain better honey yields, have healthier and therefore stronger colonies and reduce the rate of absconding.

Shorter foraging ranges of the Eastern honeybee should be considered when estimating pollination services. Relying on abundant knowledge from the Western honeybees might lead to wrong predictions about supply and demand on pollination services in the tropics of South Asia. Gene flow between forest fragments and agricultural areas is also dependent on the distances that bees will negotiate. Being the main pollinator group, it is important to consider their species-specific foraging ranges that might differ significantly from bees in temperate climates. Most crops and wild plants in the Asian tropics depend on bee pollination, and as such getting realistic estimates of pollination services and gene flow across the landscape is significant for both food security and income generation. A recent census has shown that nearly 70% of the Indian population is dependent on agriculture for their livelihood and many millions of people around the globe rely on the food produced (Chandra and Malaya 2011).

This study has shown that there are geographic, intraspecific and interspecific variation in the foraging ranges of the *Apis* species. Therefore, more research is needed to understand the foraging behaviour of the Eastern honeybee as our knowledge derived from studies on Western honeybee data might not be applicable to tropical bees and their habitats.

References

ABOU-SHAARA, H. 2014. The foraging Behaviour of honey bees, *Apis mellifera*: a review. Veterinarni Medicina, 59.

ABOU-SHAARA, H. F., AL-GHAMDI, A. A. & MOHAMED, A. A. 2013. Honey bee colonies performance enhance by newly modified beehives. Journal of Apicultural Science, 57, 45.

ADLAKHA, R. & DHALIWAL, H. 1979. Insect pollination of seed cauliflower (*Brassica oleracea var. botrytis*) with special reference to the role of honey bees. Indian Bee Journal.

ADLER, L. S. 2000. The ecological significance of toxic nectar. Oikos, 91, 409-420.

AI, H. 2009. Vibration-processing interneurons in the honeybee brain. Frontiers in Systems Neuroscience, 3.

ALM, J., OHNMEISS, T. E., LANZA, J. & VRIESENGA, L. 1990. Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. Oecologia, 84, 53-57.

ALY, A. & HASSAN, A. 1999. Foraging preference of honeybees for pollen of some maize varieties in middle Egypt. Shashpa, 6, 125-136.

AMDAM, G. V., AASE, A. L. T., SEEHUUS, S.-C., FONDRK, M. K., NORBERG, K. & HARTFELDER, K. 2005. Social reversal of immunosenescence in honey bee workers. Experimental Gerontology, 40, 939-947.

AMDAM, G. V., RUEPPELL, O., FONDRK, M. K., PAGE, R. E. & NELSON, C. M. 2009. The nurse's load: early-life exposure to brood-rearing affects Behaviour and lifespan in honey bees (*Apis mellifera*). Experimental Gerontology, 44, 467-471.

AMENT, S. A., CORONA, M., POLLOCK, H. S. & ROBINSON, G. E. 2008. Insulin signalling is involved in the regulation of worker division of labour in honey bee colonies. Proceedings of the National Academy of Sciences, 105, 4226-4231.

ARENAS, A., FERNÁNDEZ, V. M. & FARINA, W. M. 2008. Floral scents experienced within the colony affect long-term foraging preferences in honeybees. Apidologie, 39, 714-722.

ARIAS, M. C. & SHEPPARD, W. S. 2005. Phylogenetic relationships of honey bees (Hymenoptera: Apinae: Apini) inferred from nuclear and mitochondrial DNA sequence data. Molecular Phylogenetics and Evolution, 37, 25-35.

BAKER, H. G. 1977. Non-sugar chemical constituents of nectar. Apidologie, 8, 349-356.

BAKER, H. G., OPLER, P. A. & BAKER, I. 1978. A comparison of the amino acid complements of floral and extrafloral nectars. Botanical Gazette, 139, 322-332.

BAKKER, D. R. 2001. Foraging and habitat selection by two species of honey bee near Lore Lindu National Park in Sulawesi, Indonesia, University of Guelph.

BALL, B. & ALLEN, M. 1988. The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. Annals of Applied Biology, 113, 237-244.

BALL, D. W. 2007. The chemical composition of honey. Journal of Chemical Education, 84, 1643.

BEEKMAN, M. & RATNIEKS, F. 2000. Long-range foraging by the honey-bee, *Apis mellifera L*. Functional Ecology, 14, 490-496.

BEEKMAN, M., SUMPTER, D., SERAPHIDES, N. & RATNIEKS, F. 2004. Comparing foraging Behaviour of small and large honey-bee colonies by decoding waggle dances made by foragers. Functional Ecology, 18, 829-835.

BERENS, P. 2009. CircStat: a MATLAB toolbox for circular statistics. Journal of Statistical Software, 31, 1-21.

BIESMEIJER, J.C., ROBERTS, S.P., REEMER, M., OHLEMÜLLER, R., EDWARDS, M., PEETERS, T., SCHAFFERS, A.P., POTTS, S.G., KLEUKERS, R., THOMAS, C.D. & SETTELE, J., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, 313(5785), pp.351-354.

BLATT, J. & ROCES, F. 2002. The control of the proventriculus in the honeybee (*Apis mellifera carnica L.*) I. A dynamic process influenced by food quality and quantity? Journal of Insect Physiology, 48, 643-654.

BLAŽYTĖ-ČEREŠKIENĖ, L., VAITKEVIČIENĖ, G., VENSKUTONYTĖ, S. & BŪDA, V. 2010. Honey bee foraging in spring oilseed rape crops under high ambient temperature conditions. Žemdirbystė= Agriculture, 97, 61-70.

BOCH, R., 1957. Rassenmässige unterschiede bei den tänzen der honigbiene (*Apis mellifica L*.). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 40(3), pp.289-320.

BOECKING, O., RATH, W. & DRESCHER, W. 1993. Behavioural strategies of *Apis mellifera* and *Apis cerana* against *Varroa jacobsoni*. International Journal of Acarology, 19, 173-177.

BORTOLOTTI, L., MONTANARI, R., MARCELINO, J., MEDRZYCKI, P., MAINI, S. & PORRINI, C. 2003. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. Bulletin of Insectology, 56, 63-67.

BROUWERS, E., EBERT, R. & BEETSMA, J. 1987. Behaviour al and physiological aspects of nurse bees in relation to the composition of larval food during caste differentiation in the honeybee. Journal of Apicultural Research, 26, 11-23.

BUCHWALD, R., BREED, M. D., GREENBERG, A. R. & OTIS, G. 2006. Interspecific variation in beeswax as a biological construction material. Journal of Experimental Biology, 209, 3984-3989.

BÜCHLER, R., DRESCHER, W. & TORNIER, I. 1992. Grooming Behaviour of *Apis cerana*, *Apis mellifera* and *Apis dorsata* and its effect on the parasitic *mites Varroa jacobson*i and*Tropilaelaps clareae*. Experimental and Applied Acarology, 16, 313-319.

CALDERONE, N. W. & JOHNSON, B. R. 2002. The within-nest Behaviour of honeybee pollen foragers in colonies with a high or low need for pollen. Animal Behaviour, 63, 749-758.

CAMAZINE, S., VISSCHER, P., FINLEY, J. & VETTER, R. 1999. House-hunting by honey bee swarms: collective decisions and individual behaviors. Insectes Sociaux, 46, 348-360.

CAPALDI, E. A. & DYER, F. C. 1999. The role of orientation flights on homing performance in honeybees. Journal of Experimental Biology, 202, 1655-1666.

CAPALDI, E. A., SMITH, A. D., OSBORNE, J. L. & FAHRBACH, S. E. 2000. Ontogeny of orientation flight in the honeybee revealed by harmonic radar. Nature, 403, 537.

CARRECK, N. L., OSBORNE, J. L., CAPALDI, E. A. & RILEY, J. R. 1999. Tracking bees with radar. Bee World, 80, 124-131.

CHAPMAN, R.E., WANG, J. & BOURKE, A.F.G., 2003. Genetic analysis of spatial foraging patterns and resource sharing in bumble bee pollinators. Molecular Ecology, 12(10), pp.2801-2808.

CHANDRA, D. G. & MALAYA, D. B. Role of e-agriculture in rural development in Indian context. Emerging Trends in Networks and Computer Communications (ETNCC), 2011 International Conference on, 2011. IEEE, 320-323.

CHINH, T. X., BOOT, W. J. & SOMMEIJER, M. J. 2005. Production of reproductives in the honey bee species *Apis cerana* in northern Vietnam. Journal of Apicultural Research, 44, 41-48.

COLLETT, T. S. & BARON, J. 1994. Biological compasses and the coordinate frame of landmark memories in honeybees. Nature, 368, 137-140.

CONNOR, L., RINDERER, T., SYLVESTER, H. & WONGSIRI, S. Asian apiculture. Proceedings of the First International Conference on the Asian Honey Bees and Bee Mites, 1993.

CORBET, S.A., FUSSELL, M., AKE, R., FRASER, A., GUNSON, C., SAVAGE, A. & SMITH, K., 1993. Temperature and the pollinating activity of social bees. Ecological Entomology, 18(1), pp.17-30.

CORLETT, R. T. 2011. Honeybees in natural ecosystems. Honeybees of Asia. Springer.

CORONA, M., ESTRADA, E. & ZURITA, M. 1999. Differential expression of mitochondrial genes between queens and workers during caste determination in the honeybee *Apis mellifera*. Journal of Experimental Biology, 202, 929-938.

COUVILLON, M. J., PEARCE, F. C. R., ACCLETON, C., FENSOME, K. A., QUAH, S. K., TAYLOR, E. L. & RATNIEKS, F. L. 2015. Honey bee foraging distance depends on month and forage type. Apidologie, 46, 61-70.

COUVILLON, M.J., SCHÜRCH, R. & RATNIEKS, F.L., 2014. Waggle dance distances as integrative indicators of seasonal foraging challenges. *PloS one*, *9*(4), p.e93495. CRANE, E. 2004. A short history of knowledge about honey bees (*Apis*) up to 1800. Bee World, 85, 6-11.

CRESSWELL, J.E., OSBORNE, J.L. & GOULSON, D., 2000. An economic model of the limits to foraging range in central place foragers with numerical solutions for bumblebees. Ecological Entomology, 25(3), pp.249-255.

DE CAMARGO, J. M. & MELLO, M. 1970. Anatomy and histology of the genital tract, spermatheca, spermathecal duct and glands of *Apis mellifica* queens (Hymenoptera: Apidae). Apidologie, 1, 351-373.

DECOURTYE, A., DEVILLERS, J., AUPINEL, P., BRUN, F., BAGNIS, C., FOURRIER, J. & GAUTHIER, M. 2011. Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. Ecotoxicology, 20, 429-437.

DEGEN, J., KIRBACH, A., REITER, L., LEHMANN, K., NORTON, P., STORMS, M., KOBLOFSKY, M., WINTER, S., GEORGIEVA, P. B. & NGUYEN, H. 2015. Exploratory Behaviour of honeybees during orientation flights. Animal Behaviour, 102, 45-57.

DELFINADO-BAKER, M., RATH, W. & BOECKING, O. 1992. Phoretic bee mites and honeybee grooming behaviour. International journal of acarology, 18, 315-322.

DEVKOTA, F. & THAPA, R. 2005. Foraging preference of *Apis cerana F*. and *Apis mellifera L*. to broccoli under caged and open conditions in Chitwan. Journal of the Institute of Agriculture and Animal Science, 26, 167-168.

DHALIWAL, H. & SHARMA, P. 1974. Foraging range of the Indian honeybee. Journal of Apicultural Research, 13, 137-141.

DRELLER, C. & KIRCHNER, W. 1993. How honeybees perceive the information of the dance language. Naturwissenschaften, 80, 319-321.

DRELLER, C. & KIRCHNER, W. 1994. Hearing in the Asian honeybees *Apis dorsata* and *Apis florea*. Insectes Sociaux, 41, 291-299.

DUAY, P., DE JONG, D. & ENGELS, W. 2003. Weight loss in drone pupae (*Apis mellifera*) multiply infested by *Varroa destructor* mites. Apidologie, 34, 61-65.

DUJARDIN, F. 1853. Quelques Observations sur les Abeilles et Particulièrement sur les Actes qui, chez ces Insectes Peuvent être Rapportès à l'Intelligence. Annales des Sciences Naturelles, Zoologie, 18, 231-240.

DYER, F. C. 1985a. Mechanisms of dance orientation in the Asian honey bee *Apis florea* L. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioural Physiology, 157, 183-198.

DYER, F. C. 1985b. Nocturnal orientation by the Asian honey bee, *Apis dorsata*. Animal Behaviour, 33, 769-774.

DYER, F. C. 1987a. Memory and sun compensation by honey bees. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioural Physiology, 160, 621-633.

DYER, F. C. 1987b. New perspectives on the dance orientation of the Asian honeybees. Neurobiology and Behaviour of Honeybees. Springer, Berlin, Heidelberg.

DYER, F.C., 2002. The biology of the dance language. Annual Review of Entomology, 47(1), pp.917-949.

DYER, F. C. & DICKINSON, J. A. 1994. Development of sun compensation by honeybees: How partially experienced bees estimate the sun's course. Proceedings of the National Academy of Sciences, 91, 4471-4474.

DYER, F. C. & SEELEY, T. D. 1991. Dance dialects and foraging range in three Asian honey bee species. Behavioral Ecology and Sociobiology, 28, 227-233.

EDRICH, W. 1977. Interaction of light and gravity in the orientation of the waggle dance of honey bees. Animal Behaviour, 25, 342-363.

EICKWORT, G. C. 1994. Evolution and life-history patterns of mites associated with bees. Mites. Springer.

EMERY, J. 1875. Ants and bees. Nature, London, 12, 25-26.

ENGEL, M. S. 1999. The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae; *Apis*).

ENGEL, M. S. 2001. A monograph of the Baltic amber bees and evolution of the Apoidea (Hymenoptera). Bulletin of the American Museum of natural History, 1-192.

ESCH, H. 1961. über die Schallerzeugung beim Werbetanz der Honigbiene. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behaviour al Physiology, 45, 1-11.

ESCH, H. 1962. Über die Auswirkung der Futterplatzqualität auf die Schallerzeugung im Werbetanz der Honigbiene. Verhandlungen der Deutschen Zoologischen Gesellschaft, 26, 302-309.

ESCH, H. 2012. Foraging honey bees: how foragers determine and transmit information about feeding site locations. Honeybee Neurobiology and Behaviour. Springer.

ESCH, H.E. & BURNS, J.E., 1995. Honeybees use optic flow to measure the distance of a food source. Naturwissenschaften, 82(1), pp.38-40.

ESCH, H. & BURNS, J. 1996. Distance estimation by foraging honeybees. Journal of Experimental Biology, 199, 155-162.

ESCH, H., GOLLER, F. & BURNS, J. 1994. Honeybee waggle dances: the “energy hypothesis” and thermoregulatory Behaviour of foragers. Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 163, 621-625.

ESCH, H. E., ZHANG, S., SRINIVASAN, M. V. & TAUTZ, J. 2001. Honeybee dances communicate distances measured by optic flow. Nature, 411, 581.

EVANS, J. D. & WHEELER, D. E. 1999. Differential gene expression between developing queens and workers in the honey bee, *Apis mellifera*. Proceedings of the National Academy of Sciences, 96, 5575-5580.

FAHRENHOLZ, L., LAMPRECHT, I. & SCHRICKER, B. 1989. Thermal investigations of a honey bee colony: thermoregulation of the hive during summer and winter and heat production of members of different bee castes.

Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology, 159, 551-560.

FARINA, W. M., GRÜTER, C. & ARENAS, A. 2012. Olfactory information transfer during recruitment in honey bees. Honeybee Neurobiology and Behaviour. Springer.

FARINA, W. M., GRÜTER, C. & DÍAZ, P. C. 2005. Social learning of floral odours inside the honeybee hive. Proceedings of the Royal Society of London B: Biological Sciences, 272, 1923-1928.

FEWELL, J. H. & WINSTON, M. L. 1992. Colony state and regulation of pollen foraging in the honey bee, *Apis mellifera L*. Behavioral Ecology and Sociobiology, 30, 387-393.

FEWELL, J. H. & WINSTON, M. L. 1996. Regulation of nectar collection in relation to honey storage levels by honey bees, *Apis mellifera*. Behavioral Ecology, 7, 286-291.

FISHER, N. I. 1995. Statistical analysis of circular data, Cambridge University Press.

FREE, J. 1957. The food of adult drone honeybees (*Apis mellifera*). The British Journal of Animal Behaviour, 5, 7-11.

FREE, J. 1958. The ability of worker honeybees (*Apis mellifera*) to learn a change in the location of their hives. Animal Behaviour, 6, 219-223.

FREE, J. B. 1970. Insect pollination of crops. Academic Press, London, England

FREE, J., FERGUSON, A. & SIMPKINS, J. R. 1985. Influence of virgin queen honeybees (*Apis mellifera*) on queen rearing and foraging. Physiological Entomology, 10, 271-274.

FRIES, I., HUAZHEN, W., WEI, S. & JIN, C. S. 1996. Grooming Behaviour and damaged mites (*Varroa jacobsoni*) in *Apis cerana cerana* and *Apis mellifera ligustica*. Apidologie, 27, 3-11.

FRIIS, E.M., PEDERSEN, K.R. & CRANE, P.R., 2006. Cretaceous angiosperm flowers: innovation and evolution in plant reproduction. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *232*(2), pp.251-293.

GARIBALDI, L.A., STEFFAN-DEWENTER, I., WINFREE, R., AIZEN, M.A., BOMMARCO, R., CUNNINGHAM, S.A., KREMEN, C., CARVALHEIRO, L.G., HARDER, L.D., AFIK, O. AND BARTOMEUS, I., 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. Science, 339(6127), pp.1608-1611.

GARY, N. 1963. Observations of mating Behaviour in the honeybee. Journal of Apicultural Research, 2, 3-13.

GARY, N. 1967. Diurnal variations in the intensity of flight activity from honeybee colonies. Journal of Apicultural Research, 6, 65-68.

GIL, M. & DE MARCO, R. J. 2005. Olfactory learning by means of trophallaxis in *Apis mellifera*. Journal of Experimental Biology, 208, 671-680.

GOLLER, F. & ESCH, H. 1990. Waggle dances of honey bees. Naturwissenschaften, 77, 594-595.

GONZÁLEZ-VARO, J.P., BIESMEIJER, J.C., BOMMARCO, R., POTTS, S.G., SCHWEIGER, O., SMITH, H.G., STEFFAN-DEWENTER, I., SZENTGYÖRGYI, H., WOYCIECHOWSKI, M. & VILÀ, M., 2013. Combined effects of global change pressures on animal-mediated pollination. Trends in Ecology & Evolution, 28(9), pp.524-530.

GOODMAN, L. 2003. Form and function in the honey bee. Form and function in the honey bee.

GOOGLE EARTH 7.1, 2017. Thiruvananthapuram and surrounding area 8°31.639'N, 76°56.070'E, elevation 29M, https://earth.google.com/web/@8.69116921,76.88938319,30.21542389a,113491.94478644d,35y,0h,0t,0r/data=CksaSRJDCiUweDNiMDVkYjgxNmQ5MTE2MmQ6MHhlYzUzYzk2NGMzOGRhOWE2GaBInLqXqCFAIbws3KgEO1NAKghLYWRha2thbBgBIAE [Accessed 3 September 2017].

GOOGLE MAPS, 2017. Map of India. [online]. Google. Available from: https://www.google.co.uk/maps/place/India/@20.0105127,64.4523315,4z/data=!3m1!4b1!4m5!3m4!1s0x30635ff06b92b791:0xd78c4fa1854213a6!8m2!3d20.593684!4d78.96288 [Accessed 3 September 2017].

GOULD, J. L. 1975. Honey bee recruitment: the dance-language controversy. Science, 189, 685-693.

GOULD, J. L. 1980. Sun compensation by bees. Science, 207, 545-547.

GOULD, J. L. 1982. Why do honey bees have dialects? Behavioral Ecology and Sociobiology, 10, 53-56.

GOULD, J. L. & TOWNE, W. F. 1987. Evolution of the dance language. The American Naturalist, 130, 317-338.

GREENLEAF, S. S., WILLIAMS, N. M., WINFREE, R. & KREMEN, C. 2007. Bee foraging ranges and their relationship to body size. Oecologia, 153, 589-596.

GRIES, M. & KOENIGER, N. 1996. Straight forward to the queen: pursuing honeybee drones (*Apis mellifera L.*) adjust their body axis to the direction of the queen. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioural Physiology, 179, 539-544.

GRÜTER, C. & FARINA, W. M. 2009. The honeybee waggle dance: can we follow the steps? Trends in Ecology & Evolution, 24, 242-247.

GUO, X., SU, S., SKOGERBOE, G., DAI, S., LI, W., LI, Z., LIU, F., NI, R., GUO, Y. & CHEN, S. 2013. Recipe for a busy bee: microRNAs in Honey Bee caste determination. PLoS One, 8, e81661.

HADISOESILO, S., OTIS, G. W. & MEIXNER, M. 1995. Two distinct populations of cavity-nesting honey bees (Hymenoptera: Apidae) in South Sulawesi, Indonesia. Journal of the Kansas Entomological Society, 399-407.

HALDANE, J. 1955. Aristotle's Account of Bees' 'Dances'. The Journal of Hellenic Studies, 75, 24-25.

HALL, K. 2017. Waggle dances, *Apis cerana*, video recording, YouTube, < https://www.youtube.com/watch?v=azt33wi51W8> [Accessed 4 September 2017].

HAYDAK, M. H. 1970. Honey bee nutrition. Annual Review of Entomology, 15, 143-156.

HE, X., WANG, W., QIN, Q., ZENG, Z., ZHANG, S. & BARRON, A. B. 2013. Assessment of flight activity and homing ability in Asian and European honey bee species, *Apis cerana* and *Apis mellifera*, measured with radio frequency tags. Apidologie, 44, 38-51.

HENRY, M., BERTRAND, C., LE FÉON, V., REQUIER, F., ODOUX, J.-F., AUPINEL, P., BRETAGNOLLE, V. & DECOURTYE, A. 2014. Pesticide risk assessment in free-ranging bees is weather and landscape dependent. Nature Communications, 5, 4359.

HEPBURN, H. R., SMITH, D. R., RADLOFF, S. E. & OTIS, G. W. 2001. Infraspecific categories of *Apis cerana*: morphometric, allozymal and mtDNA diversity. Apidologie, 32, 3-23.

HEPPERLE, C. & HARTFELDER, K. 2001. Differentially expressed regulatory genes in honey bee caste development. Naturwissenschaften, 88, 113-116.

HERAN, H. 1956. Ein Beitrag zur Frage nach der Wahrnehmungsgrundlage der Entfernungsweisung der Bienen (*Apis mellifica L.*). Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioural Physiology, 38, 168-218.

HERBERT JR, E. W. & SHIMANUKI, H. 1978. Chemical composition and nutritive value of bee-collected and bee-stored pollen. Apidologie, 9, 33-40.

HONEYBEE GENOME SEQUENCING CONSORTIUM, 2006. Insights into social insects from the genome of the honeybee *Apis mellifera*. Nature, 443(7114), p.931.

HOWELL, D. C. 2012. Statistical methods for psychology, Cengage Learning.

HUANG, Z.-Y., ROBINSON, G. & BORST, D. 1994. Physiological correlates of division of labour among similarly aged honey bees. Journal of Comparative Physiology A, 174, 731-739.

HUANG, Z.-Y. & ROBINSON, G. E. 1996. Regulation of honey bee division of labour by colony age demography. Behavioral Ecology and Sociobiology, 39, 147-158.

INOUE, T. & SALMAH, S. 1990. Nest site selection and reproductive ecology of the Asian honey bee, *Apis cerana indica*, in central Sumatra. Nest site selection and reproductive ecology of the Asian honey bee, *Apis cerana indica*, in central Sumatra., 219-232.

JOHNSON, R.N., OLDROYD, B.P., BARRON, A.B. AND CROZIER, R.H., 2002. Genetic control of the honey bee (*Apis mellifera*) dance language: segregating dance forms in a backcrossed colony. Journal of Heredity, 93(3), pp.170-173.

JOHNSON, D. & WENNER, A. 1970. Recruitment efficiency in honeybees: studies on the role of olfaction. Journal of Apicultural Research, 9, 13-18.

JONES, J. C., MYERSCOUGH, M. R., GRAHAM, S. & OLDROYD, B. P. 2004. Honey bee nest thermoregulation: diversity promotes stability. Science, 305, 402-404.

JOSHI, N.C. & JOSHI, P.C., 2010. Foraging behaviour of *Apis* spp. on apple flowers in a subtropical environment. New York Science Journal, 3(3), pp.71-76.

KARLSSON, T. 1990. The natural nest of the Asian hive bee (*Apis cerana*) in Bangladesh: a minor field study. The natural nest of the Asian hive bee (*Apis cerana*) in Bangladesh: a minor field study.

KEVAN, P. G. & MENZEL, R. 2012. The plight of pollination and the interface of neurobiology, ecology and food security. The Environmentalist, 32, 300-310.

KIM, Y. S. & SMITH, B. H. 2000. Effect of an amino acid on feeding preferences and learning Behaviour in the honey bee, *Apis mellifera*. Journal of Insect Physiology, 46, 793-801.

KIRCHNER, W., DRELLER, C., GRASSER, A. & BAIDYA, D. 1996. The silent dances of the Himalayan honeybee, *Apis laboriosa*. Apidologie, 27, 331-339.

KIRCHNER, W. H. & DRELLER, C. 1993. Acoustical signals in the dance language of the giant honeybee, *Apis dorsata*. Behavioral Ecology and sociobiology, 33, 67-72.

KIRCHNER, W. H. & SOMMER, K. 1992. The dance language of the honeybee mutant diminutive wings. Behavioral Ecology and sociobiology, 30, 181-184.

KLEIN, A.M., VAISSIERE, B.E., CANE, J.H., STEFFAN-DEWENTER, I., CUNNINGHAM, S.A., KREMEN, C. AND TSCHARNTKE, T., 2007. Importance of pollinators in changing landscapes for world crops. Proceedings of the Royal Society of London B: Biological Sciences, 274(1608), pp.303-313.

KOENIGER, G., KOENIGER, N. & PHIANCHAROEN, M. 2011. Comparative reproductive biology of honeybees. Honeybees of Asia. Springer.

KOENIGER, N., KOENIGER, G., PUNCHIHEWA, R., FABRITIUS, M. & FABRITIUS, M. 1982. Observations and experiments on dance communication in *Apis florea* in Sri Lanka. Journal of Apicultural Research, 21, 45-52.

KOENIGER, N., KOENIGER, G. & WIJAYAGUNASEKARA, N. H. 1981. Beobachtungen über die Anpassung von *Varroa jacobsoni* an ihren natürlichen Wirt *Apis cerana* in Sri Lanka. Apidologie, 12, 37-40.

KOETZ, A. H. 2013. Ecology, Behaviour and control of *Apis cerana* with a focus on relevance to the Australian incursion. Insects, 4, 558-592.

KRALJ, J. & FUCHS, S. 2006. Parasitic *Varroa destructor* mites influence flight duration and homing ability of infested *Apis mellifera* foragers. Apidologie, 37, 577-587.

KRALJ, J. & FUCHS, S. 2010. *Nosema* sp. influences flight Behaviour of infected honey bee (*Apis mellifera*) foragers. Apidologie, 41, 21-28.

KSHIRSAGAR, K. & RANADE, D. 1981. Morphometric characterization of Indian hive bee *Apis cerana indica* F.(Apidae, Hymenoptera), worker. Journal of University Poona Science and Technology 54, 101-120.

KÖRNER, I. 1940. Zeitgedächtnis und Alarmierung bei den Bienen. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 27, 445-459.

LANDGRAF, T., ROJAS, R., NGUYEN, H., KRIEGEL, F. & STETTIN, K., 2011. Analysis of the waggle dance motion of honeybees for the design of a biomimetic honeybee robot. PloS one, 6(8), p.e21354.

LINANDER, N., HEMPEL DE IBARRA, N. & LASKA, M. 2012. Olfactory detectability of L-amino acids in the European honeybee (*Apis mellifera*). Chemical Senses, 37, 631-638.

LINDAUER, M. 1956. Über die Verständigung bei indischen Bienen. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 38, 521-557.

LINDAUER, M. 1967. Recent advances in bee communication and orientation. Annual Review of Entomology, 12, 439-470.

LINDAUER, M. 1971. The functional significance of the honeybee waggle dance. The American Naturalist, 105, 89-96.

LOPER, G. M. & TAYLOR JR, O. R. 1987. Detection and monitoring of honeybee drone congregation areas by radar. Apidologie, 18, 163-172.

ŁOPUCH, S. & TOFILSKI, A. 2017. Direct visual observation of wing movements during the honey bee waggle dance. Journal of Insect Behavior, 30, 199-210.

MATTU, V. & VERMA, L. 1985. Studies on the annual foraging cycle *of Apis cerana* indica F. in Simla hills of Northwest Himalayas. Apidologie, 16, 1-18.

MENZEL, R., GREGGERS, U., SMITH, A., BERGER, S., BRANDT, R., BRUNKE, S., BUNDROCK, G., HÜLSE, S., PLÜMPE, T., SCHAUPP, F. AND SCHÜTTLER, E., 2005. Honey bees navigate according to a map-like spatial memory. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(8), pp.3040-3045.

MENZEL, R., DE MARCO, R. J. & GREGGERS, U. 2006. Spatial memory, navigation and dance Behaviour in *Apis mellifera*. Journal of Comparative Physiology A, 192, 889-903.

MICHELSEN, A. 2003. Signals and flexibility in the dance communication of honeybees. Journal of Comparative Physiology A, 189, 165-174.

MICHELSEN, A., ANDERSEN, B. B., STORM, J., KIRCHNER, W. H. & LINDAUER, M. 1992. How honeybees perceive communication dances, studied by means of a mechanical model. Behavioral Ecology and Sociobiology, 30, 143-150.

MICHELSEN, A., TOWNE, W. F., KIRCHNER, W. H. & KRYGER, P. 1987. The acoustic near field of a dancing honeybee. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioural Physiology, 161, 633-643.

MICHENER, C.D., 1974. The social behavior of the bees: a comparative study (Vol. 73, No. 87379). Harvard University Press.

MICHENER, C.D., 2000. The bees of the world (Vol. 1). Johns Hopkins Press.

MISHRA, R. & GARG, R. 1998. Perspectives in Indian apiculture.

MISHRA, R. & KUMAR, Y. 1997. *Apis mellifera L*. for Indian apiculture. Perspective in Indian apiculture, 119-149.

MORITZ, R. F. & FUCHS, S. 1998. Organization of honeybee colonies: characteristics and consequences of a superorganism concept. Apidologie, 29, 7-21.

NAYAK, K.G. & DAVIDAR, P., 2010. Pollinator limitation and the effect of breeding systems on plant reproduction in forest fragments. Acta Oecologica, 36(2), pp.191-196.

NEPI, M., SOLIGO, C., NOCENTINI, D., ABATE, M., GUARNIERI, M., CAI, G., BINI, L., PUGLIA, M., BIANCHI, L. & PACINI, E. 2012. Amino acids and protein profile in floral nectar: much more than a simple reward. Flora-Morphology, Distribution, Functional Ecology of Plants, 207, 475-481.

NEVARD, L., SOMANATHAN H., HEMPEL DE IBARRA, N. 2016. Foraging behaviour of social bees in the South Asain tropics. Unpublished poster presentation at: 16th congress of the International Society for Behavioral Ecology, July 28-August 3 2016, Exeter, United Kingdom.

NICHOLLS, E. & HEMPEL DE IBARRA, N. 2017. Assessment of pollen rewards by foraging bees. Functional Ecology, 31, 76-87.

NICOLSON, S. W. 2009. Water homeostasis in bees, with the emphasis on sociality. Journal of Experimental Biology, 212, 429-434.

NICOLSON, S. W. 2011. Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. African Zoology, 46, 197-204.

NÚÑEZ, J.A., 1966. Quantitative Beziehungen zwischen den Eigenschaften von Futterquellen und dem Verhalten von Sammelbienen. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 53(2), pp.142-164.

NÚÑEZ, J., 1970. The relationship between sugar flow and foraging and recruiting behaviour of honey bees (Apis mellifera L.). Animal Behaviour, 18, pp.527-538.

NÚÑEZ, J.A., 1982. Honeybee foraging strategies at a food source in relation to its distance from the hive and the rate of sugar flow. Journal of Apicultural Research, 21(3), pp.139-150.

OLDROYD, B. P., HALLING, L. A., GOOD, G., WATTANACHAIYINGCHAROEN, W., BARRON, A. B., NANORK, P., WONGSIRI, S. & RATNIEKS, F. L. 2001. Worker policing and worker reproduction in *Apis cerana*. Behavioral Ecology and Sociobiology, 50, 371-377.

OLDROYD, B. P. & WONGSIRI, S. 2006. Asian honey bees: biology, conservation, and human interactions, Harvard University Press.

OSBORNE, J., CLARK, S., MORRIS, R., WILLIAMS, I., RILEY, J., SMITH, A., REYNOLDS, D. & EDWARDS, A. 1999. A landscape-scale study of bumble bee foraging range and constancy, using harmonic radar. Journal of Applied Ecology, 36, 519-533.

PARTAP, U. & PARTAP, T. 1997. Managed crop pollination: the missing dimension of mountain productivity.

PARTAP, U., SHUKLA, A. & VERMA, L. Comparative foraging Behaviour of *Apis cerana* and *Apis mellifera* in pollinating peach and plum flowers in Kathmandu Valley Nepal. Proceedings of the 4th Asian apicultural association conference, Kathmandu, 2000.

PARTAP, U. & VERMA, L. 1994. Pollination of radish by *Apis cerana*. Journal of Apicultural Research, 33, 237-241.

PENG, Y.-S., FANG, Y., XU, S. & GE, L. 1987. The resistance mechanism of the Asian honey bee*, Apis cerana Fabr*., to an ectoparasitic mite, *Varroa jacobsoni* Oudemans. Journal of Invertebrate Pathology, 49, 54-60.

PERNAL, S. F. & CURRIE, R. W. 2001. The influence of pollen quality on foraging Behaviour in honeybees (*Apis mellifera L*.). Behavioral Ecology and Sociobiology, 51, 53-68.

PILLING, E., CAMPBELL, P., COULSON, M., RUDDLE, N. & TORNIER, I. 2013. A four-year field program investigating long-term effects of repeated exposure of honey bee colonies to flowering crops treated with thiamethoxam. PLoS one, 8, e77193.

POTTS, S.G., BIESMEIJER, J.C., KREMEN, C., NEUMANN, P., SCHWEIGER, O. & KUNIN, W.E., 2010. Global pollinator declines: trends, impacts and drivers. Trends in Ecology & Evolution, 25(6), pp.345-353.

PUNCHIHEWA, R., KOENIGER, N., KEVAN, P. & GADAWSKI, R. 1985. Observations on the dance communication and natural foraging ranges of *Apis cerana*, *Apis dorsata* and *Apis florea* in Sri Lanka. Journal of Apicultural Research, 24, 168-175.

RADLOFF, S. E., HEPBURN, C., HEPBURN, H. R., FUCHS, S., HADISOESILO, S., TAN, K., ENGEL, M. S. & KUZNETSOV, V. 2010. Population structure and classification of *Apis cerana*. Apidologie, 41, 589-601.

RAMESH. A., SOMANATHAN H., HEMPEL DE IBARRA, N. 2016. Foraging division of labour in the Asian honeybee, *Apis cerana*. Unpublished poster presentation at: 16th congress of the International Society for Behavioral Ecology, July 28-August 3 2016, Exeter, United Kingdom.

RAMIREZ-ROMERO, R., CHAUFAUX, J. & PHAM-DELÈGUE, M.-H. 2005. Effects of Cry1Ab protoxin, deltamethrin and imidacloprid on the foraging activity and the learning performances of the honeybee *Apis mellifera*, a comparative approach. Apidologie, 36, 601-611.

REDDY, C. C. 1980. Observations on the annual cycle of foraging and brood rearing by *Apis cerana indica* colonies. Journal of Apicultural Research, 19, 17-20.

REINHARD, J. & SRINIVASAN, M. V. 2009. The role of scents in honey bee foraging and recruitment. Food exploitation by social insects: ecological, behavioural, and theoretical approaches, 1, 165-182.

REINHARD, J., SRINIVASAN, M. V., GUEZ, D. & ZHANG, S. W. 2004. Floral scents induce recall of navigational and visual memories in honeybees. Journal of Experimental Biology, 207, 4371-4381.

REYES-CARRILLO, J. L., EISCHEN, F. A., CANO-RIOS, P., RODRIGUEZ-MARTINEZ, R. & NAVA CAMBEROS, U. 2007. Pollen collection and honey bee forager distribution in cantaloupe. Acta Zoológica Mexicana, 23, 29-36.

REYNOLDS, A. M., SMITH, A. D., REYNOLDS, D. R., CARRECK, N. L. & OSBORNE, J. L. 2007. Honeybees perform optimal scale-free searching flights when attempting to locate a food source. Journal of Experimental Biology, 210, 3763-3770.

RIESSBERGER, U. & CRAILSHEIM, K. 1997. Short-term effect of different weather conditions upon the Behaviour of forager and nurse honey bees (*Apis mellifera carnica* Pollmann). Apidologie, 28, 411-426.

RILEY, J. & SMITH, A. 2002. Design considerations for an harmonic radar to investigate the flight of insects at low altitude. Computers and Electronics in Agriculture, 35, 151-169.

RILEY, J. R., GREGGERS, U., SMITH, A. D., REYNOLDS, D. R. & MENZEL, R. 2005. The flight paths of honeybees recruited by the waggle dance. Nature, 435, 205.

RINDERER, T. & BEAMAN, L. 1995. Genic control of honey bee dance language dialect. Theoretical and Applied Genetics, 91, 727-732.

RINDERER, T., KOENIGER, N., TINGEK, S., MARDAN, M. & KOENIGER, G. 1989. A morphological comparison of the cavity dwelling honeybees of Borneo *Apis koschevnikovi* (Buttel-Reepen, 1906) and *Apis cerana* (Fabricius, 1793). Apidologie, 20, 405-411.

RINDERER, T., OLDROYD, B., WONGSIRI, S., SYLVESTER, H., DE GUZMAN, L., STELZER, J. & RIGGIO, R. 1995. A morphological comparison of the dwarf honey bees of southeastern Thailand and Palawan, Philippines. Apidologie, 26, 387-394.

RINDERER, T. E., WONGSIRI, S., KUANG, B., LIU, J., OLDROYD, B. P., SYLVESTER, H. A. & DE GUZMAN, L. 1996. Comparative nest architecture of the dwarf honey bees. Journal of Apicultural Research, 35, 19-26.

ROBINSON, G. E. 1992. Regulation of division of labour in insect societies. Annual Review of Entomology, 37, 637-665.

ROBINSON, G. E., PAGE, R. E., STRAMBI, C. & STRAMBI, A. 1992. Colony integration in honey bees: mechanisms of behavioural reversion. Ethology, 90, 336-348.

ROD-IM, P., DUANGPHAKDEE, O., RADLOFF, S., PIRK, C. W., HEPBURN, C. & HEPBURN, R. 2015. Azimuth-dependent waggle dances; flight and foraging activities of the red dwarf honeybee, *Apis florea Fabricius* (1787). Journal of Apicultural Research, 54, 246-254.

ROHRSEITZ, K., 1998. *Biophysikalische und ethologische Aspekte der Tanzkommunikation der Honigbienen (Apis mellifera carnica Pollm.)*. Shaker.

ROHRSEITZ, K. & TAUTZ, J. 1999. Honey bee dance communication: waggle run direction coded in antennal contacts? Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 184, 463-470.

ROUBIK, D.W., 1992. *Ecology and natural history of tropical bees*. Cambridge University Press.

ROUBIK, D. W., SAKAGAMI, S. F. & KUDO, I. 1985. A note on distribution and nesting of the Himalayan honey bee *Apis laboriosa* Smith (Hymenoptera: Apidae). Journal of the Kansas Entomological Society, 746-749.

RUEPPELL, O., BACHELIER, C., FONDRK, M. K. & PAGE, R. E. 2007. Regulation of life history determines lifespan of worker honey bees (*Apis mellifera L.*). Experimental Gerontology, 42, 1020-1032.

RUTTNER, F. 1988. Biogeography and taxonomy of honeybees Springer. Berlin, Germany.

RUTTNER, F. & VOLPRECHT, M. 1983. Experimental analysis of reproductive interspecies isolation of *Apis mellifera L*. and *Apis cerana* Fabr. Apidologie, 14, 309-327.

SAKAGAMI, S. F. 1960. Preliminary report on the specific difference of Behaviour and other ecological characters between European and Japanese honeybees. Acta Hymenopterologica, 1, 171.

SAKAGAMI, S. F. & AKAHIRA, Y. 1958. Comparison of ovarian size and number of ovarioles between the workers of Japanese and European honeybees: Studies on the Japanese honeybee, *Apis indica cerana Fabricius*. I. 昆蟲, 26, 103-109.

SARMA, M. S., ESCH, H. & TAUTZ, J. 2004. A comparison of the dance language in *Apis mellifera carnica* and *Apis florea* reveals striking similarities. Journal of Comparative Physiology A, 190, 49-53.

SASAKI, M., VEERESH, G.K., MALLIK, B. AND VIRAKTAMATH, C.A., 1990. Absconding dance in *Apis cerana japonica*: a slow and long tail-waggling motivates the whole colony to translocate. 11th Int. Congr., International Union for the Study of Social Insects, Bangalore, Oxford Press and IBH, New Delhi, pp.125-126.

SCHNEIDER, S.S. & MCNALLY, L.C., 1992. Factors influencing seasonal absconding in colonies of the African honey bee, *Apis mellifera scutellata*. Insectes Sociaux, *39*(4), pp.403-423.

SCHNEIDER, C. W., TAUTZ, J., GRÜNEWALD, B. & FUCHS, S. 2012. RFID tracking of sublethal effects of two neonicotinoid insecticides on the foraging Behaviour of *Apis mellifera*. PloS one, 7, e30023.

SCHNEIDER, S. S. 1989. Spatial foraging patterns of the African honey bee, *Apis mellifera scutellata*. Journal of Insect Behavior, 2, 505-521.

SCHRICKER, B., 1974. Der einfluss subletaler dosen von Parathion (E 605) auf die Entfernungsweisung bei der Honigbiene. Apidologie, 5(2), pp.149-175.

SCHULZ, D. J., BARRON, A. B. & ROBINSON, G. E. 2002. A role for octopamine in honey bee division of labour. Brain, Behaviour and Evolution, 60, 350-359.

SCHWEIGER, E. M. 1958. Über individuelle Unterschiede in der Entfernungs- und Richtungsangabe bei den Tänzen der Bienen. Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 41, 272-299.

SEELEY, T. 1995. The wisdom of the hive Cambridge. MA: Harvard University Press.

SEELEY, T. D. 1989. The honey bee colony as a superorganism. American Scientist, 77, 546-553.

SEELEY, T. D., MIKHEYEV, A. S. & PAGANO, G. J. 2000. Dancing bees tune both duration and rate of waggle-run production in relation to nectar-source profitability. Journal of Comparative Physiology A, 186, 813-819.

SEELEY, T. D., SEELEY, R. H. & AKRATANAKUL, P. 1982. Colony defense strategies of the honeybees in Thailand. Ecological Monographs, 52, 43-63.

SEELEY, T. D. & TOWNE, W. F. 1992. Tactics of dance choice in honey bees: do foragers compare dances? Behavioral Ecology and Sociobiology, 30, 59-69.

SHARMA, H., GUPTA, J. & THAKUR, J. Pollination studies on apple and pear. Proc. 7* Int. Conf. On Tropical Bees: Management and Diversity, Chiang Mai, Thailand, 2000. 19-25.

SI, A., SRINIVASAN, M. V. & ZHANG, S. 2003. Honeybee navigation: properties of the visually drivenodometer9. Journal of Experimental Biology, 206, 1265-1273.

SILVA, D., MOISAN-DE-SERRES, J., SOUZA, D., HILGERT-MOREIRA, S., FERNANDES, M., KEVAN, P. & FREITAS, B. 2013. Efficiency in pollen foraging by honey bees: Time, motion, and pollen depletion on flowers of *Sisyrinchium palmifolium*. Journal of Pollination Ecology, 11, 27-32.

SIMPSON, J. 1961. Nest climate regulation in honey bee colonies. Science, 133, 1327.

SLESSOR, K. N., WINSTON, M. L. & LE CONTE, Y. 2005. Pheromone communication in the honeybee (*Apis mellifera L*.). Journal of Chemical Ecology, 31, 2731-2745.

SMITH, D. R., VILLAFUERTE, L., OTIS, G. & PALMER, M. R. 2000. Biogeography of *Apis cerana* F. and A. nigrocincta Smith: insights from mtDNA studies. Apidologie, 31, 265-279.

SMITH, F. 1958. Communication and foraging range of African bees compared with that of European and Asian bees. Bee World, 39, 249-252.

SNODGRASS, R. E. 1984. Anatomy of the honey bee, Cornell University Press.

SPANGLER, H. G. 1991. Do honey bees encode distance information into the wing vibrations of the waggle dance? Journal of Insect Behavior, 4, 15-20.

SPITZNER, J. E. 1788. Ausführliche Beschreibung der Korbbienenzucht im sächsischen Churkreise, ihrer Dauer und ihres Nutzens, ohne künstliche Vermehrung, Junius.

SRINIVASAN, M., ZHANG, S. & BIDWELL, N. 1997. Visually mediated odometry in honeybees. Journal of Experimental Biology, 200, 2513-2522.

SRINIVASAN, M. V. 2014. Going with the flow: a brief history of the study of the honeybee's navigational 'odometer'. Journal of Comparative Physiology A, 200, 563-573.

SRINIVASAN, M. V., ZHANG, S., ALTWEIN, M. & TAUTZ, J. 2000. Honeybee Navigation: Nature and Calibration of the" Odometer". Science, 287, 851-853.

STEFFAN-DEWENTER, I. & KUHN, A. 2003. Honeybee foraging in differentially structured landscapes. Proceedings of the Royal Society of London B: Biological Sciences, 270, 569-575.

SU, S., CAI, F., SI, A., ZHANG, S., TAUTZ, J. & CHEN, S. 2008. East learns from West: Asiatic honeybees can understand dance language of European honeybees. PLoS One, 3, e2365.

SUSHIL, S., STANLEY, J., HEDAU, N. & BHATT, J. 2013. Enhancing seed production of three Brassica vegetables by honey bee pollination in north-western Himalayas of India. Universal Journal of Agricultural Research, 1, 49-53.

SUWANNAPONG, G., BENBOW, M. & NIEH, J. 2012. Biology of Thai honeybees: natural history and threats.

SYLVESTER, H. A., LIMBIPICHAI, K., WONGSIRI, S., RINDERER, T. E. & MARDAN, M. 1998. Morphometric studies of *Apis cerana* in Thailand and the Malaysian peninsula. Journal of Apicultural Research, 37, 137-145.

TAN, K., YANG, M., RADLOFF, S., HEPBURN, H., ZHANG, Z., LUO, L. & LI, H. 2008. Dancing to different tunes: heterospecific deciphering of the honeybee waggle dance. Naturwissenschaften, 95, 1165-1168.

TAN, K., YANG, S., WANG, Z.-W., RADLOFF, S. E. & OLDROYD, B. P. 2012. Differences in foraging and broodnest temperature in the honey bees *Apis cerana* and *A. mellifera*. Apidologie, 43, 618-623.

TANAKA, H., ROUBIK, D., KATO, M., LIEW, F. & GUNSALAM, G. 2001. Phylogenetic position of *Apis nuluensis* of northern Borneo and phylogeography of *A. cerana* as inferred from mitochondrial DNA sequences. Insectes Sociaux, 48, 44-51.

TANNER, D. & VISSCHER, K. 2009. Does the body orientation of waggle dance followers affect the accuracy of recruitment? Apidologie, 40, 55-62.

TANNER, D. A. & VISSCHER, P. K. 2008. Do honey bees average directions in the waggle dance to determine a flight direction? Behavioral Ecology and Sociobiology, 62, 1891-1898.

TAUTZ, J. 1996. Honeybee waggle dance: recruitment success depends on the dance floor. Journal of Experimental Biology, 199, 1375-1381

TENCZAR, P., LUTZ, C. C., RAO, V. D., GOLDENFELD, N. & ROBINSON, G. E. 2014. Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. Animal Behaviour, 95, 41-48.

THE ASTRONOMICAL APPLICATIONS DEPARTMENT OF THE U.S. NAVAL OBSERVATORY, 2015, Sun or Moon Altitude/Azimuth Table, viewed 3 August 2017, http://aa.usno.navy.mil/data/docs/AltAz.php.

THOM, C., GILLEY, D. C., HOOPER, J. & ESCH, H. E. 2007. The scent of the waggle dance. PLoS Biology, 5, e228.

THOMPSON, H., COULSON, M., RUDDLE, N., WILKINS, S. & HARKIN, S. 2016. Thiamethoxam: Assessing flight activity of honeybees foraging on treated oilseed rape using radio frequency identification technology. Environmental Toxicology and Chemistry, 35, 385-393.

TOWNE, W. F. 1985. Acoustic and visual cues in the dances of four honey bee species. Behavioral Ecology and Sociobiology, 16, 185-187.

TOWNE, W. F. & GOULD, J. L. 1988. The spatial precision of the honey bees' dance communication. Journal of Insect Behavior, 1, 129-155.

VAN GEYSTELEN, A., BENAETS, K., DE GRAAF, D. C., LARMUSEAU, M. & WENSELEERS, T. 2016. Track-a-Forager: a program for the automated analysis of RFID tracking data to reconstruct foraging Behaviour. Insectes Sociaux, 63, 175-183.

VERMA, L. & DULTA, P. 1986. Foraging Behaviour of *Apis cerana indica* and *Apis mellifera* in pollinating apple flowers. Journal of Apicultural Research, 25, 197-201.

VERMA, L. & PARTAP, U. 1994. Foraging Behaviour of *Apis cerana* on cauliflower and cabbage and its impact on seed production. Journal of Apicultural research, 33, 231-236.

VERMA, L. & RANA, R. 1994. Further studies on the Behaviour of *Apis cerana* and *Apis mellifera* foraging on apple flowers. Journal of Apicultural Research, 33, 175-179.

VIJAYAN, S., HEMPEL DE IBARRA, N., SOMANATHAN H. 2016. Ontogeny pf orientation flights in the Eastern honeybee, *Apis cerana*. Unpublished poster presentation at: 16th congress of the International Society for Behavioral Ecology, July 28-August 3 2016, Exeter, United Kingdom.

VISSCHER, P. K. & SEELEY, T. D. 1982. Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology, 63, 1790-1801.

VISWANATHAN, G. M., BULDYREV, S. V., HAVLIN, S. & DA LUZ, M. 1999. Optimizing the success of random searches. Nature, 401, 911.

VON BUTTEL-REEPEN, H. 1906. Contributions to the systematics, biology as well as the historical and geographical distribution of honeybees (Apis mellifica L.) their variability and other Apis species. Mitteilungen aus dem Zoologischen Museum in Berlin, 3, 117-201.

VON FRISCH, K., 1923. Ein Zwergwels, der kommt, wenn man ihm pfeift. Biologisches Zentralblatt, *43*, pp.439-446.

VON FRISCH, K. 1946. Die Tänze der Bienen: mit 16 Abb, Springer.

VON FRISCH, K. 1951. Orientierungsvermögen und sprache der bienen. Naturwissenschaften, 38, 105-112.

VON FRISCH, K. 1955. The dancing bees. Harcourt, Brace.

VON FRISCH, K. 1967. The dance language and orientation of bees. Harvard University Press, Cambridge

WADDINGTON, K. D., HERBERT, T. J., VISSCHER, P. K. & RICHTER, M. R. 1994. Comparisons of forager distributions from matched honey bee colonies in suburban environments. Behavioral Ecology and Sociobiology, 35, 423-429.

WADDINGTON, K.D., Nelson, C.M. & Page, R.E., 1998. Effects of pollen quality and genotype on the dance of foraging honey bees. Animal Behaviour, *56*(1), pp.35-39.

WAPPLER, T., LABANDEIRA, C.C., ENGEL, M.S., ZETTER, R. AND GRÍMSSON, F., 2015. Specialized and generalized pollen-collection strategies in an ancient bee lineage. Current Biology, 25(23), pp.3092-3098.

WEBSTER, T. C. & DELAPLANE, K. S. 2001. Mites of the honey bee, Dadant & Sons.

WEIDENMÜLLER, A. & TAUTZ, J. 2002. In-Hive Behaviour of Pollen Foragers (*Apis mellifera*) in Honey Bee Colonies Under Conditions of High and Low Pollen Need. Ethology, 108, 205-221.

WEISS, K. 1967. Zur vergleichenden Gewichtsbestimmung von Bienenköniginnen. Zur Bienenfauna, 9, 1-21.

WENNER, A. M. 1962. Sound production during the waggle dance of the honey bee. Animal Behaviour, 10, 79-95.

WENNER, A. M., WELLS, P. H. & JOHNSON, D. L. 1969. Honey bee recruitment to food sources: olfaction or language? Science, 164, 84-86.

WHEELER, D., BUCK, N. & EVANS, J. D. 2006. Expression of insulin pathway genes during the period of caste determination in the honey bee, *Apis mellifera*. Insect Molecular Biology, 15, 597-602.

WILLIAMS, I. H. & CHRISTIAN, D. 1991. Observations on *Phacelia tanacetifolia Bentham* (Hydrophyllaceae) as a food plant for honey bees and bumble bees. Journal of Apicultural Research, 30, 3-12.

WINSTON, M. L. 1991. The biology of the honey bee, Harvard University Press.

WOYKE, J., 1980. Evidence and action of cannibalism substance in *Apis cerana indica*. *Journal of Apicultural research*, *19*(1), pp.6-16.

WOYKE, J. 1992. Diurnal flight activity of African bees *Apis mellifera* adansonii in different seasons and zones of Ghana. Apidologie, 23, 107-117.

WOYKE, J. Differences in body colour expression between European and Asian honeybees. Proceedings of 4th Asian Apicultural Association Conference, Kathmandu, 2000. 20-23.

WRIGHT, G., BAKER, D., PALMER, M., STABLER, D., MUSTARD, J., POWER, E., BORLAND, A. & STEVENSON, P. 2013. Caffeine in Floral Nectar Enhances a Pollinators Memory of Reward. Science, 339, 1202-1204.

YANG, E., CHUANG, Y., CHEN, Y. & CHANG, L. 2008. Abnormal foraging Behaviour induced by sublethal dosage of imidacloprid in the honey bee (Hymenoptera: Apidae). Journal of Economic Entomology, 101, 1743-1748.

YUCEL, B. & DUMAN, I., 2005. Effects of foraging activity of honeybees (*Apis mellifera L.*) on onion (*Allium cepa*) seed production and quality. Pakistan Journal of Biological Sciences, 8(1), pp.123-126.